Vol. 32, No. 4 November 1945

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF APPLIED BIOLOGISTS

BY

W. B. BRIERLEY

AND

R. W. MARSH

WITH THE ASSISTANCE OF

F. C. BAWDEN H. MARTIN

J. W. EVANS I. THOMAS

CAMBRIDGE UNIVERSITY PRESS
LONDON: BENTLEY HOUSE
CHICAGO: The University of Chicago Press
(Agents for the United States)
BOMBAY, CALCUTTA, MADRAS: Macmillan

All rights reserved

PRINTED IN GREAT BRITAIN

The Association of Applied Biologists

President: C. B. WILLIAMS, M.A., Sc.D.

Vice-Presidents: PROF. W. BROWN, M.A., D.Sc., F.R.S. AND W. C. MOORE, M.A.

Hon. Treasurer
W. C. MOORE, M.A.
Plant Pathology Laboratory,
Milton Road, Harpenden, Herts.

Hon. Editor
PROF. W. B. BRIERLEY, D.Sc.
University of Reading,
Berks.

Hon. Assistant Editor
R. W. MARSH, M.A.
Research Station,
Long Ashton, Bristol.

Hon. Secretary
W. P. K. FINDLAY, D.Sc.
Forest Products Research Laboratory,
Princes Risborough, Bucks.

Hon. Assistant Secretary
H. SHAW, M.Sc., PH.D.
East Malling Research Station,
Nr. Maidstone, Kent.

Council

L. A. ALLEN, D.Sc.
G. M. BATES, D.Sc.
F. C. BAWDEN, M.A.
J. R. BUSVINE, PH.D.
M. COHEN, PH.D.
G. V. B. HERFORD, B.A.
J. T. MARTIN, D.Sc.
E. W. MASON, M.A.
H. W. MILES, D.Sc.
M. H. MOORE, M.Sc.
R. T. PEARL, B.Sc.
MISS A. P. WILSON, A.R.C.S.

Plant Pests and Diseases Committee

Hon. Secretary: W. G. KEYWORTH, PH.D., A.R.C.S., East Malling Research Station, Nr. Maidstone, Kent.

CONTENTS OF VOL. 32, NO. 4

PAGE

1. The influence of periodic fallowing on the prevalence of viable weed seeds in arable soil. By WINIFRED E. BRENCHLEY and KATHERINE WARINGTON. (With 5 Text-figures) 285
2. The effect of eyespot (*Cercosporella herpotrichoides* Fron.) on wheat and the influence of nitrogen on the disease. By MARY D. GLYNNE, W. M. DION and J. W. WEIL. (With Plate 9 and 6 Text-figures) 297
3. A comparison of *Fusarium avenaceum* and *Fusarium caeruleum* as causes of wastage in stored potato tubers. By F. JOAN MOORE, PH.D. (With 1 Text-figure) 304
4. The effect of disinfecting and bruising seed potatoes on the incidence of dry rot (*Fusarium caeruleum* (Lib.) Sacc.). By T. SMALL, PH.D., A.R.C.S. 310
5. Field trials of copper fungicides for the control of potato blight. I. Foliage protection and yield. By E. C. LARGE, B.Sc., A.R.I.C. (With 4 Text-figures) 319
6. Blind-seed disease of rye-grass (*Phialea temulenta* Prill. & Delacr). By E. L. CALVERT and A. E. MUSKETT. (With Plates 10 and 11 and 1 Text-figure) 329
7. The incidence and distribution of some diseases of the adult honeybee (*Apis mellifera* L.) in England and Wales. By C. G. BUTLER. (With 4 Text-figures) 344
8. Soil populations of beet eelworm (*Heterodera schachtii* Schm.) in relation to cropping. By F. G. W. JONES, M.A. (With 19 Text-figures) 351

The influence of periodic fallowing on the prevalence of viable weed seeds in arable soil

BY WINIFRED E. BRENCHLEY AND KATHERINE WARINGTON
Botanical Department, Rothamsted Experimental Station, Harpenden, Herts

(With 5 Text-figures)

In view of the favourable reduction in the weed-seed population on Broadbalk wheat field (Rothamsted Experimental Station) by the 2-year fallows between 1925 and 1929 a cyclic fallowing of one section out of five per year was adopted to maintain control over the weeds. Annual samples for determining the weed seeds were taken from plots receiving farmyard manure, minerals and sulphate of ammonia together, and minerals and sulphate of ammonia in alternate years.

The numbers of seedlings obtained were grouped to show the reduction in weed-seed population during fallow and the behaviour of various species in succeeding years. By this grouping the effect of the variable seasonal and cultivation factors is greatly reduced, as several years are included in each figure.

Most species are reduced by fallow, rise more or less rapidly during succeeding years, and are again reduced by a later fallow, usually to a lower level than by the first. *Alopecurus agrestis* showed the greatest variation from year to year. *Papaver rhoeas* was the outstanding exception to the general rule, as, once reduced by fallow, it failed to reassert itself except in a few cases, and was further reduced by the later fallow. These two species, with *Alchemilla arvensis*, *Veronica arvensis* and *Medicago lupulina*, contributed about 90 % of the total seeds.

Alternate manuring with minerals and sulphate of ammonia greatly influenced the production of weed seeds. With most species nitrogen induced heavier seed production, as was shown by the average number per section compared with those produced with minerals. *Alopecurus agrestis*, *Alchemilla arvensis*, and usually *Papaver rhoeas* responded thus, *Veronica arvensis* was the only one of the main species to behave irregularly, whereas *Medicago lupulina* showed a reverse response, being more benefited by minerals than nitrogen.

In the first year after fallowing the crop and weeds alike were usually able to benefit by the stored fertility and did not appear to interfere with one another except where farmyard manure was used, when the competition of the crop at first prevented the weed-seed population from rising. In the later years the crop tended to decrease, and the weeds to increase, until the balance was restored by another fallow.

Field observations, as well as laboratory samples, have shown a steady improvement in the cleanness of Broadbalk field, the most noticeable reduction being in poppies. The various weed species fall into definite groups, in two of which the reduction or increase in numbers induced by fallowing is maintained, while in the third there is an irregular increase and decrease in numbers over a period of years.

The general conclusion is that the fallowing of one section per year has fully justified itself, as the total weed-seed population has not only been kept in check, but has also been gradually decreased during the 15 years the system has been applied.

I. INTRODUCTION

One of the great advantages of rotation farming is that the successive crops require their own special cultivation and are sown and harvested at different times of the year. Weeds also vary greatly in habit and response to season, and the frequent changes in crop cultivation provide opportunities of attacking first one, and then another, troublesome weed at a vulnerable period in its life history. Under these conditions a bare fallow for 12 months is seldom necessary as a method of weed control.

In special circumstances, as on several experimental fields at Rothamsted, the same crop may be grown for years in succession; e.g. Broadbalk field has carried wheat for over 100 years without any crop rotation. As the wheat is autumn sown, weed control presents many difficulties, and on occasion drastic measures have to be used. The undue prevalence of field poppy (*Papaver rhoeas*) and black bent (*Alopecurus agrestis*) dictated a system of sectional fallowing from 1926 to 1929, after which the whole field was again in wheat in 1930. During this period a long-term experiment was instituted to follow the changes in the weed-seed population of the soil, and full results have been published (Brenchley & Warington, 1930, 1933, 1936; Warington, 1936).

By the time of the 1930 harvest three points were fully established:

(1) Fallowing brings about various degrees of reduction in the seed population of different weeds

in the soil. Some species, e.g. *Alopecurus agrestis*, may be reduced to a very small percentage even after one year's fallow, whereas others, e.g. *Capsella bursa-pastoris*, *Arenaria serpyllifolia* and *Veronica* spp., are much less affected and may even be increased in number if the fallowing operations have been faulty.

(2) Some species, e.g. *Papaver rhoeas*, when once reduced by fallow, tend to remain more or less at the new level, and do not usually regain their former position. This behaviour is independent of the degree to which fallowing effects reduction.

Other species, e.g. *Alopecurus agrestis*, however drastically they have been reduced by fallowing, reassert themselves quickly, and in the course of 2–3 years may even be much more plentiful than before the fallow.

(3) The first crop of wheat after fallow is usually heavy and considerably above the normal, thus introducing a greater factor of crop competition than under ordinary conditions. Some weed species respond to this increased competition and remain at the low level to which fallowing has reduced them, but others, e.g. *Alopecurus agrestis*, *Veronica hederaefolia*, *Stellaria media*, etc., increase in spite of the heavier wheat crop. This may be due to the habit of the weeds, by which they can re-establish themselves while the wheat is still small, or to their ability to make full use of the increased store of fertility in the soil, thus encouraging strong plants able to withstand the competition of the wheat.

These results suggested that periodic fallowing for one year in every five might be effective in keeping the weeds within bounds by checking the continued increase of the most prevalent species. To prevent undue interference with the crop yields it was decided to fallow one section per year, making a 5-years' cycle, starting with those that were in fallow at harvest 1926 and 1927 (Table 1).

TABLE 1. *Fallowing scheme for all plots*

Section	1st period	2nd period	3rd period
1	1926–7	1931	1936
2	1926–7	1932	1937
5	1928–9	1933	1938
4	1928–9	1934	1939
3	1926–9	1935	1940

As it was necessary, after 1930, to reduce the scale of the laboratory side of the work, samples were taken only from three of the seven plots originally examined. The selection was made to compare the results with organic manure (plot 2) and with complete artificials (plot 7, sulphate of ammonia and minerals). A third plot (plot 18) was included owing to the usual prevalence of such special weeds as *Medicago lupulina*, *Euphorbia exigua* and *Polygonum aviculare*. This plot received ammonium sulphate and mineral manures in alternate years, the nitrogen being applied in the odd years, 1931, 1933, etc. This proved to be a very fortunate selection, as information was obtained from the alternate manuring which did not appear in the other cases. The method of sampling and after-treatment was identical with that already described (Brenchley & Warington, 1930, pp. 238–41).

In 1930, the year before the cyclic fallowing began, the number of viable weed seeds in the soil of different sections varied in response to the previous fallowing treatment. Sections 1 and 2, after 3 years' recovery, showed a higher total than sections 3 and 4 with only 1 year's recovery, while section 3, which had been fallowed for 4 years, was naturally much behind (Table 2).

TABLE 2. *Weed-seed population in* 1930. *Plots* 2, 7, 18 *together; all species.* 3 *sq.ft. per section*

Section		
1	11,614	Fallowed 1931, 1936, 1941
2	12,252	,, 1932, 1937, 1942
5	7,701	,, 1933, 1938, 1943
4	8,982	,, 1934, 1939, 1944
3	3,576	,, 1935, 1940, 1945

(Sections arranged in order of fallowing.)

This initial variation did not affect the consideration of the results, which were either dealt with by sections or by whole plots in which all the variations were incorporated.

II. Reduction of seed population in soil by fallowing

The degree of effectiveness of fallowing chiefly depends upon correct cultivation while the land is free from the crop, though abnormal seasonal conditions at critical times may also affect the results. When the crop is harvested many weeds are still in active growth and will continue to stock the soil with fresh seed unless they are destroyed. Therefore ploughing as soon as possible after harvest is the first essential. If ploughing is delayed for any length of time, the actual seed population of the soil for certain species may be greater after a year in fallow than before. This was well shown in the earlier part of the experiment when one area of the field, supposed to be under fallow, was not ploughed until the March after the harvest (Brenchley & Warington, 1936, pp. 480–2).

The practical value of fallowing may be considered from several aspects:

(1) The immediate reduction in the weed-seed population of the soil, often resulting in a decreased weed infestation in the following crop.

(2) The behaviour of the various weed species in succeeding years—i.e. whether they remain at a low level or whether they return rapidly into prominence, even becoming more plentiful than before the fallow.

(3) Effect of varying manurial treatment on numbers of buried weed seeds.

(4) The influence of fallowing upon crop yield, in relation to weed-seed population.

These alternatives are covered by the available data. For convenience and to avoid repetition (1) and (2) will be considered together.

(1), (2) *Reduction in weed-seed population during fallow and the behaviour of various species in succeeding years*

The reduction in the weed-seed population brought about by fallowing depends upon a variety of factors, interacting with one another. The time of ploughing, the frequency and thoroughness of cultivation, rainfall and temperature at different periods of the year all play their part in determining the degree of reduction. This again is associated with the difference in the life history of the various weeds, as conditions which will bring about drastic reduction in some species may so encourage the seed production of others that the soil is left richer in viable seeds than before fallowing was begun (Brenchley & Warington, 1936, pp. 480–2). Any comparison of the effect of fallowing in different years is inevitably influenced by these varying conditions, but, broadly speaking, the response of the more important individual species or groups of species from year to year is similar within wide limits of variation. When an exceptional deviation occurs in either direction it is usually possible to ascertain the determining factor.

As the experiment was designed to discover the ultimate effect of the periodic fallow it is necessary, and legitimate, to obtain the total number of weed seeds per plot by summing the figures for each section in its year of fallow, and again for each succeeding year until the next fallowing. By doing this the effects of the variable factors, such as time of ploughing, intervals between cultivations and the effect of rainfall, sunshine, frost and drought are greatly reduced, as five successive seasons are represented in each total. In discussing the data obtained the actual numbers of seeds are shown in the graphs, whereas the tables give the percentage figures based on the number for the year before the first fallowing, which is reckoned as 100. These percentages are expressed to the nearest whole number. One complete sequence from fallow to fallow is considered, as this gives the 5-year figures (one year per section) for each point on the curve. To get this one sequence results have to be drawn over a period of 11 years (1930–40) to include the years before fallow. The grouping is set out in Table 3.

TABLE 3. *Grouping of sections at same stage of treatment*

			Year after fallow				
Section	Year before fallow	Fallow	1st	2nd	3rd	4th	Fallow
1	1930	1931	1932	1933	1934	1935	1936
2	1931	1932	1933	1934	1935	1936	1937
3	1934	1935	1936	1937	1938	1939	1940
4	1933	1934	1935	1936	1937	1938	1939
5	1932	1933	1934	1935	1936	1937	1938

TABLE 4. *All species: percentage*

			Year after fallow				
	Year before fallow	Fallow	1st	2nd	3rd	4th	Fallow
Plot 2	100	48	46	59	64	79	32
,, 7	100	46	68	80	91	85	30
,, 18	100	55	108	103	108	104	48
All plots	100	49	69	77	85	87	35

(*a*) *All species taken together.* The total weed-seed population of the soil was reduced to about one-half by the first fallowing, with slight variations from plot to plot. This was followed by a rise, rapid at first, decreasing in rate in succeeding years, but failing to attain the original level before the second fallow caused another drastic reduction (Table 4 and Fig. 1 C).

With farmyard manure (plot 2) the initial rise was delayed for a year, but then a steady rise occurred until the next fallow which reduced the population below that left after the previous fallow. On plot 18, with the alternate nitrogen and mineral manuring, the behaviour was quite different and called for explanation. This will be discussed in a later section (II (3)) of the paper after the individual species have been considered.

(*b*) *Alopecurus agrestis.* The rise and fall of this species is more spectacular than any other, owing to its ready response to fallowing and its rapid resumption of its original status. The increase after

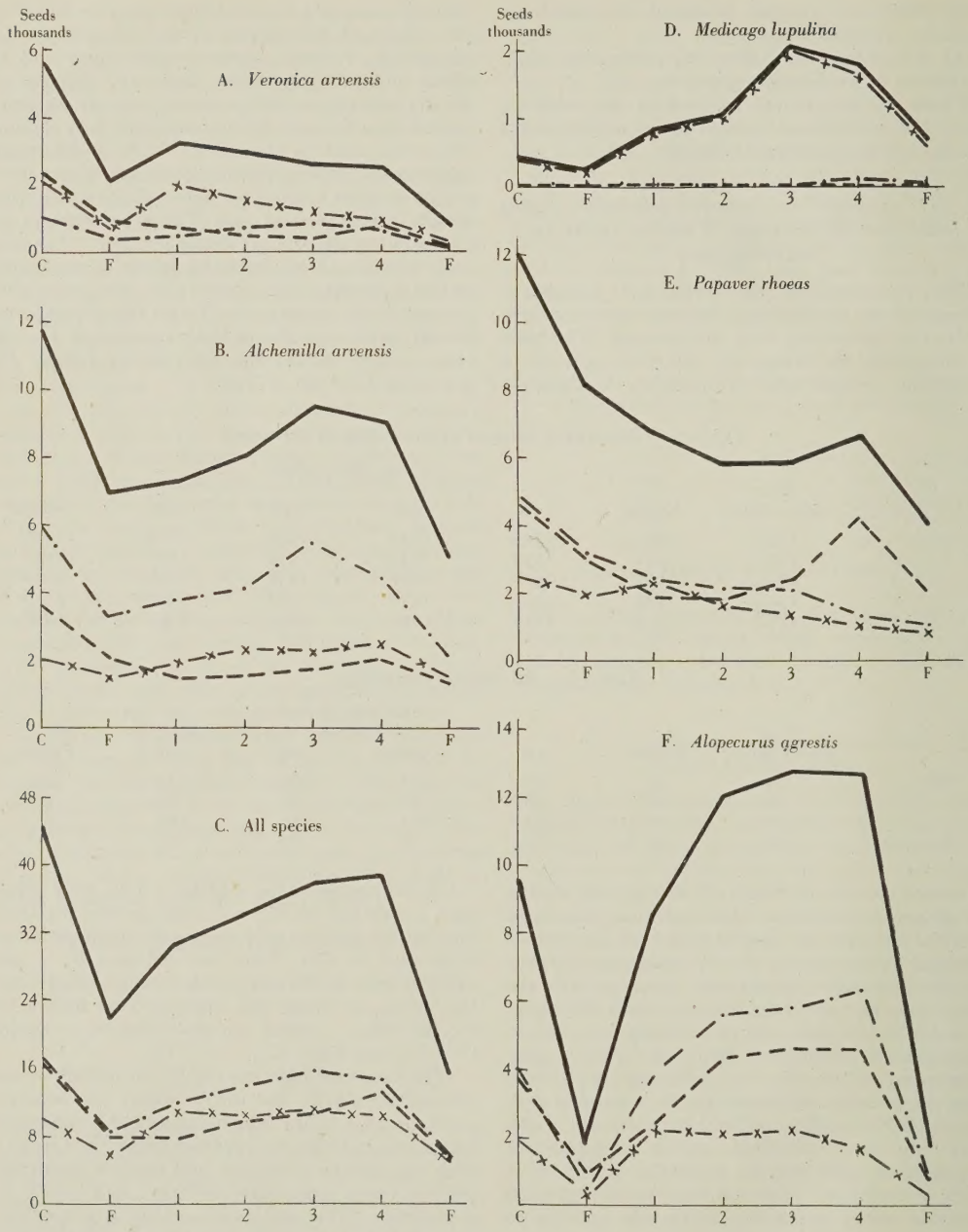


Fig. 1. Sums of viable seeds for all five sections for fallowed period and succeeding years.

C = year before fallow.
F = fallow year.
1, 2, 3, 4 = years after fallow.

——— All plots, 15 sq.ft.
– – – – – Plot 2, 5 sq.ft.
–·–·–·– Plot 7, 5 sq.ft.
–×–×–×– Plot 18, 5 sq.ft.

fallowing is so pronounced that the initial soil population may be exceeded in 1 or 2 years. After this, relatively little change occurs until a further cut by fallowing (Table 5 and Fig. 1 F), apart from a slight tendency to increase on plots 2 and 7 and to decrease on plot 18.

(*c*) *Papaver rhoeas*. The response of field poppy, once the outstandingly dominant weed of the field, is totally different from that of any other species present. The reduction by fallow is not very drastic, only approximately one-third, but the after-effect varies considerably from plot to plot.

out in the 5-year averages. With field poppy, on the contrary, the difference in the figures was relatively slight except for big increases in 1935 on sections 1 and 2 of plot 2 (Table 7). Consequently the 5-year means containing these latter figures were heavily weighted, causing the sharp rise in the proportion of field poppy with farmyard manure shown in Table 6, Fig. 1 E, for the third and fourth year after fallowing. No explanation can be offered for the abnormal behaviour of field poppy on this particular area, in view of the lack of a similar response with other species under

TABLE 5. Alopecurus agrestis: *percentage*

	Year before fallow	Fallow	Year after fallow 1st	2nd	3rd	4th	Fallow
Plot 2	100	25	65	115	124	123	21
,, 7	100	14	96	139	145	157	19
,, 18	100	16	118	111	119	89	16
All plots	100	19	89	125	132	131	19

TABLE 6. Papaver rhoeas: *percentage*

	Year after fallow	Fallow	Year after fallow 1st	2nd	3rd	4th	Fallow
Plot 2	100	64	41	40	53	91	45
,, 7	100	65	51	44	42	28	21
,, 18	100	78	93	73	56	41	35
All plots	100	67	56	49	49	55	33

TABLE 7. *Irregular behaviour of* Papaver rhoeas *compared with rest of species in* 1934 *and* 1935

	Papaver rhoeas						Rest of species					
	Plot 2		Plot 7		Plot 18		Plot 2		Plot 7		Plot 18	
Section	*1934*	*1935*	*1934*	*1935*	*1934*	*1935*	*1934*	*1935*	*1934*	*1935*	*1934*	*1935*
1	358	2192	136	141	105	211	880	1750	1341	2802	1602	2820
2	519	1110	467	656	315	311	757	2028	1071	3849	1204	2884
3	66	(57)	363	(334)	205	(147)	753	(391)	1004	(445)	1119	(622)
4	(634)	290	(612)	368	(429)	350	(712)	1179	(894)	2538	(730)	3105
5	456	362	464	452	302	330	352	844	879	3249	641	3001

Figures in brackets for fallowed sections.

With minerals and nitrogen together (plot 7) a steady reduction continued year by year, with a further drop due to the later fallow, leaving the population at only one-fifth of its original numbers. With minerals and sulphate of ammonia alternately there was a slight rise for the first year after fallow, with a subsequent steady decrease as with minerals and nitrogen together. With farmyard manure (plot 2) an initial drop occurred, with a big rise in the third and fourth years (Table 6, Fig. 1 E).

The year 1935 was weedy, following a very clean year, and a heavy increase in weed seeds, excluding field poppy, was evident on *all* sections under crop on the three plots, so that the uplift was levelled

identical conditions. The marked contrast between the response of *Papaver rhoeas* and the other species to the particular conditions of the experiment is best seen in Fig. 2, considering the three plots together.

(*d*) *Alchemilla arvensis*. This low-growing, almost insignificant species provided a surprisingly large proportion of the total population of weed seeds, frequently exceeding *Papaver rhoeas* in number for corresponding periods (Table 8).

After the first fallowing the general tendency was for a rise for 3–4 years, with a further drop due to the second fallow. With farmyard manure reduction continued during the first year in crop and the later rise was very gradual. With minerals and nitrogen

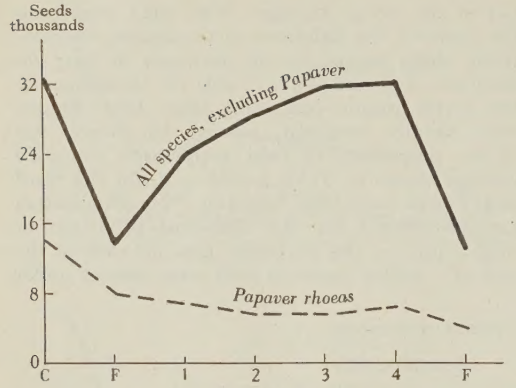


Fig. 2. Sums of viable seeds for all five sections for fallowed period and succeeding years. *C* = year before fallow. *F* = fallow year. 1, 2, 3, 4 = years after fallow. All plots together, 15 sq.ft.

The reduction due to the first fallow was less, the species more than regaining its original position during the 4 years under crop, while the second fallow left the population identical with that of the first. This difference is lost when all three plots are considered together, as the numbers of *Alchemilla arvensis* on plot 18 are less than on either of the others (Fig. 1 B).

(*e*) *Veronica arvensis*. Periodic fallowing proved very effective in reducing this low-growing species, the numbers falling to 32–42 % with the first treatment and to 13–22 % with the second on the different plots (Table 9).

The response during the cropped years was somewhat variable. With minerals and nitrogen there was a steady rise till after the third year, with farmyard manure a slight fall till the fourth year, while with the alternate manuring a sharp rise occurred during the first year in crop, followed by a steady fall, hardly accentuated by the second fallow (Fig. 1 A).

TABLE 8. Alchemilla arvensis: *percentage*

	Year before fallow	Fallow	Year after fallow 1st	2nd	3rd	4th	Fallow
Plot 2	100	57	41	43	49	56	36
,, 7	100	56	63	69	90	74	36
,, 18	100	73	96	114	110	123	75
All plots	100	59	62	69	81	77	43

TABLE 9. Veronica arvensis: *percentage*

	Year before fallow	Fallow	Year after fallow 1st	2nd	3rd	4th	Fallow
Plot 2	100	41	30	24	20	37	13
,, 7	100	42	48	71	88	69	22
,, 18	100	32	92	72	55	44	13
All plots	100	37	58	52	47	46	15

TABLE 10. Medicago lupulina

Actual numbers per 5 sq.ft.

Plot	Year before fallow	Fallow	Year after fallow 1st	2nd	3rd	4th	Fallow
2	3	4	5	26	20	43	28
7	1	4	5	10	18	100	43
18	434	239	797	1019	2049	1658	607
			Percentages				
18	100	55	184	235	472	382	140

the species reasserted itself to a considerable degree, but was more reduced by the second fallow than on either of the other plots, the final percentage being the same as with farmyard manure. In both these cases the second fallow brought the percentage of weed seeds considerably below that left after the first treatment. On plot 18, however, response was again affected by the alternation of the fertilizers. In no case did the species quite regain its initial position, though it came near to doing so on plot 18 during the first cropped year.

(*f*) *Medicago lupulina*. This species was only present in any abundance on plot 18, very few plants occurring on either of the others, too few for the percentage figures to have any significance (Fig. 1 D). In Table 10, therefore, the actual number

of weed seeds is given for all plots, with the percentage for plot 18 only.

Less than half of the seeds were eliminated by fallow. This was to be expected, as the length of possible dormancy had been proved in the earlier experiment (Brenchley & Warington, 1936, p. 495) to be at least 10 years.

Under crop a rapid increase took place, raising the numbers far above those present before the first fallow, and consequently after the second fallow there were more seeds left in the soil than were present before the experiment began. Even on the other plots on which the species was almost negligible at first, a similar increase took place, the numbers rising from 3 to 43 on plot 2, and from 1 to 100 on plot 7, per 5 sq.ft.

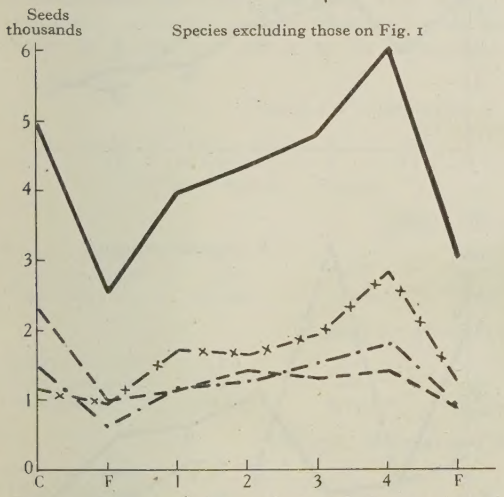


Fig. 3. Sums of viable seeds for all five sections for fallowed period and succeeding years. *C* = year before fallow. *F* = fallow year. 1, 2, 3, 4 = years after fallow. ———— All plots, 15 sq.ft. – – – – – – Plot 2, 5 sq.ft. –·–·–·– Plot 7, 5 sq.ft. –×–×–×– Plot 18, 5 sq.ft.

The behaviour of *Medicago lupulina* was unlike that of any other species, and will be discussed more fully later (see p. 293).

(*g*) *Other species*. The five species considered above accounted for nearly 40,000 buried weed seeds on the three plots together for the year before fallow, the rest, comprising at least twenty-two species, contributing only 5,000 more. After the usual reduction by fallowing a considerable increase occurred on plots 7 and 18 until the second fallow period, the rise being less marked on plot 2, where the numbers remained steady after the second year (Fig. 3). The proportion of the 'other species' to the total ranged from 7 to 14 % of the seed population, the range of variation being thus too small to affect the general changes on the field.

(3) *Effect of varying manurial treatment on numbers of buried weed seeds*

Plot 18 is the only case on Broadbalk field in which there is any change in the manuring from year to year, minerals and ammonium sulphate being given alternately. On this plot, after the usual drop in the total weed-seed population due to fallowing, the numbers of seeds regained their original level and then remained steady until the next fallow (Fig. 1 C) in contrast to the continued rise on the other plots.

With this scheme of fallowing any point on the curve in Fig. 1 C represents the sum of the population of three sections with one fertilizer and two with the other, the next point reversing the manuring for the same sections. Fig. 4 H shows the mean population per section for each fertilizer at the same stage in the fallowing cycle. After a preliminary reduction with both fertilizers due to fallowing, the number of weed seeds produced with ammonium sulphate was far larger than with minerals. A year later, on the same sections, the relative numbers were reversed, with the manuring, and similarly throughout the cycle. The same sequence was seen to occur even when the available figures were incomplete and did not cover the full 5 years. Thus, the mean number of weed seeds was always higher on the sections receiving ammonium sulphate than on those with minerals. The result of this differential action due to the reversal of manuring is that the mean soil population remained steady at the level to which it rose during the first year after fallow. The increased seed production with sulphate of ammonia is evidently due to the better growth of the weeds encouraged by nitrogen, inducing more flower and seed production on individual plants, despite the actual number of potential plants being limited by the lower number of seeds left in the soil after the previous year's mineral manures. Similarly, the reduced number of seeds produced with mineral manures must depend upon the weaker plants produced, although larger supplies of seed were present in the soil after the previous sulphate of ammonia. The reaction of *Alopecurus agrestis* and *Alchemilla arvensis* was similar, the nitrogen figures always exceeding those with minerals, the resultant giving a flat or very gradual curve (Fig. 4 G, L).

With a single exception in the first year after fallow *Papaver rhoeas* also gave a larger response to nitrogen than to minerals, but the difference between the means at any point was less marked. In this case the steady decrease in the number of *Papaver rhoeas* after the fallow is also shown by the steady fall in the analysed curves (Fig. 4 J). *Veronica arvensis* is the only one of the five main species which shows no regular response to either fertilizer, the mean ammonium sulphate figures sometimes exceeding the minerals, and vice versa (Fig. 4 K).

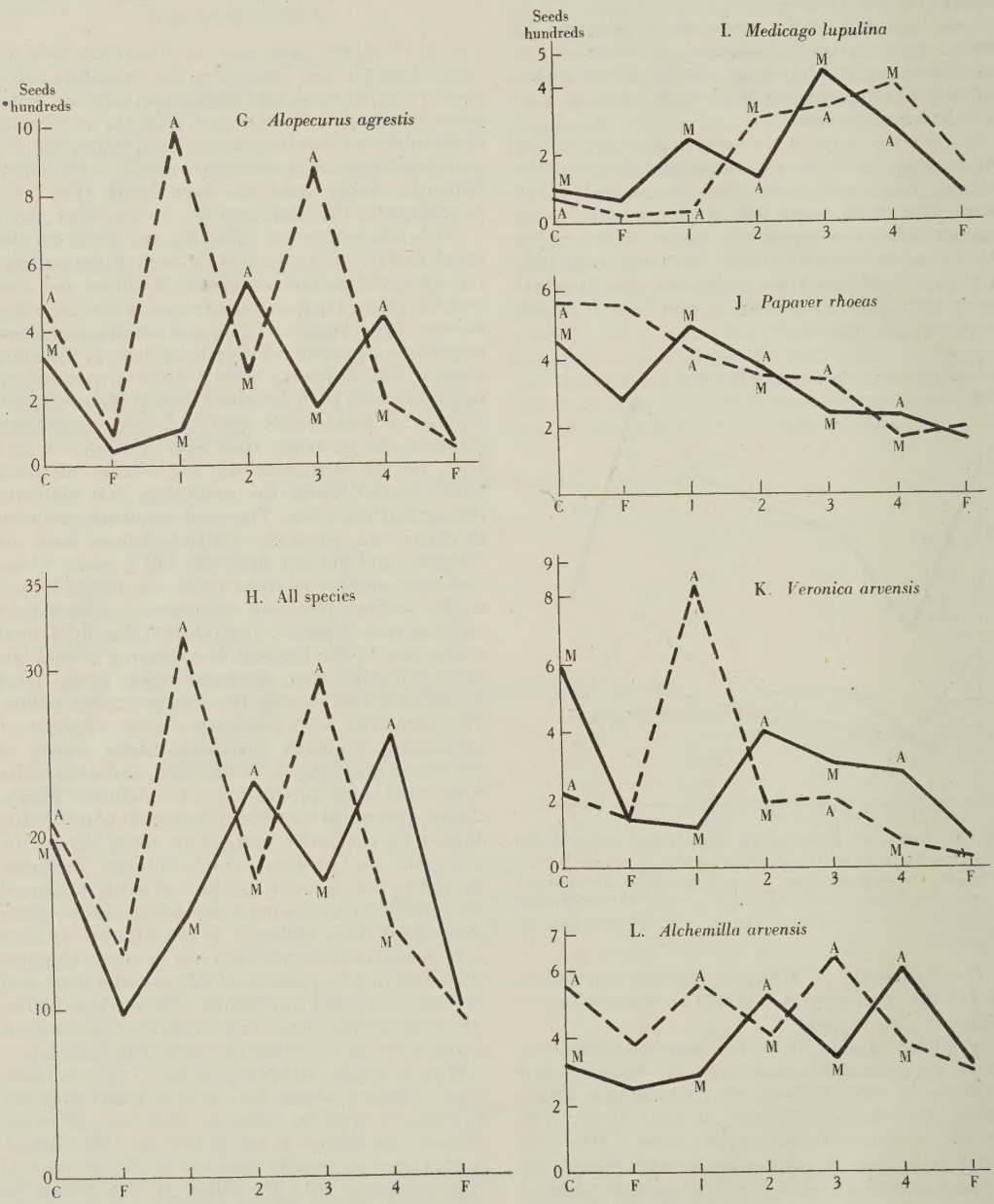


Fig. 4. Number of viable seeds for fallowed period and succeeding years, on Plot 18, with minerals and nitrogen alternately.

M = mineral manures.
A = sulphate of ammonia.

———— Mean of sections 1, 3, 5. Area 1 sq.ft.
– – – – – Mean of sections 2, 4. Area 1 sq.ft.

Medicago lupulina behaves in the reverse way to most of the others, as the mean number of seeds is increased by the minerals instead of by the ammonium sulphate (Fig. 4 I). The field records show that *Medicago lupulina* is most plentiful on the unmanured plot and on those receiving minerals, either alone or associated with a low dressing of ammonium sulphate. Plot 17, the duplicate of plot 18, but receiving the alternative manure each year, also has abundant *Medicago lupulina*, especially in the minerals year. There is thus a close association of the species with mineral manuring on this soil, which persists even if some nitrogenous manure is added, provided the latter is not at too heavy a rate. So on plots 17 and 18, the species is much more prevalent in the years with minerals, as nitrogen alone discourages its growth. This response to mineral manuring may be connected with the ability of leguminous plants to fix atmospheric nitrogen, which occurs more freely if there is little or no nitrogenous fertilizer present. A parallel case of encouragement of leguminous plants by mineral manures occurs on the Park Grass plots where *Lathyrus pratensis*, *Trifolium pratense*, *T. repens*, and *Lotus corniculatus* are present in abundance in grass cut for hay.

(4) *Influence of fallowing on crop yield, in relation to weed-seed population*

The beneficial effect of fallowing on the growth of wheat is very evident in the field, as the crop succeeding the fallow stands out in height and colour above all the rest. The effect is only obvious for the one year, the later crops being more normal in type.

The crop increase for the first year after fallow was very marked in the straw from each plot, a steady decline then setting in until the next fallow (Table 11). The grain was not greatly affected with farmyard manure, the yield rose slightly with minerals and nitrogen, but a great improvement occurred with alternate minerals and ammonium sulphate in this first year. The response in later years was less consistent than in the straw, with a further rise at some stage on plots 2 and 7, while some reduction occurred with the alternate manuring.

It might have been expected that the heavy competition of the wheat crop would prevent the weeds from re-establishing themselves immediately after fallow. This was the case with *farmyard manure* where the total competition of the crop was greatest. Here the population remained constant for a year, but later rose again, though it did not attain the level of that prior to fallowing. With *minerals and ammonium sulphate applied together* the crop competition was less severe and a rise of weed-seed population paralleled that of the wheat in the first year. After this the weed seeds increased as the crop decreased, but again they remained depressed below the pre-fallow figure. With *alternate minerals and nitrogen* the proportionate rise in weed seeds was much greater in relation to the crop increase than on the other plots, the pre-fallow level being reached at once and maintained until the second fallow again reduced the number.

TABLE 11. *Comparison of yield and weed-seed population*

	Year after fallow	Fallow	Year after fallow: 1st	2nd	3rd	4th	Fallow
			Plot 2. Farmyard manure				
Straw, cwt./acre	43·2	—	62·3	50·5	42·9	45·1	—
Grain, cwt./acre	14·1	—	14·8	14·9	16·9	14·4	—
Weed seeds	16,645	7,915	7,653	9,754	10,663	13,188	5,390
			Plot 7. Minerals + sulphate of ammonia				
Straw, cwt./acre	35·4	—	53·9	43·6	39·0	38·8	—
Grain, cwt./acre	13·9	—	15·5	16·3	17·0	14·0	—
Weed seeds	17,483	8,111	11,852	13,992	15,821	14,800	5,175
			Plot 18. Minerals and sulphate of ammonia alternately				
Straw, cwt./acre	20·3	—	41·7	31·1	24·9	30·3	—
Grain, cwt./acre	9·0	—	14·8	11·8	9·6	10·6	—
Weed seeds	10,301	5,619	11,071	10,557	11,154	10,721	4,910

It may be concluded that on some plots the extra fertility stored during the fallow is sufficient to benefit weeds and crop alike for the first year, except such cases where the extra wheat crop induced is so heavy that the competition is severe enough to prevent the weeds reasserting themselves at first.

III. CUMULATIVE EFFECT OF PERIODIC FALLOWING, 1925–40

During the period under consideration most of Broadbalk field had been under fallow for 4 years,

at different times, and one section for 6 years, of which four were consecutive. Field observations have shown a steady improvement in the cleanness of the land, the most noticeable reduction being in *Papaver rhoeas*. In 1925 much of the field was scarlet at flowering time, whereas now *Papaver rhoeas* is by no means a conspicuous weed. The number of viable weed seeds, as estimated from the samples taken on plots 2, 7, and 18, has shown a steady decrease since 1925 (Table 12 and Fig. 5).

Fig. 5 shows graphically the very great fluctuations in the weed-seed population of the soil from year to year, caused largely by seasonal conditions, coupled with the effect of the various processes of cultivation as well as of the bare fallow. The highest figure occurred in 1925, before fallowing began, and as there was no approach to this figure again for 15 years, the efficiency of the fallow is at once obvious. From 1926 to 1929 three-fifths of the field was always under fallow, and the steady reduction

TABLE 12. *Number of viable buried weed seeds at 5-year intervals. Plots 2, 7, 18 together. 15 sq.ft.*

	Actual population at harvest				Relative population in percentages of 1925 numbers			
Group A	*1925*	*1930*	*1935*	*1940*	*1925*	*1930*	*1935*	*1940*
Aethusa cynapium	141	31	13	18	100	22	9	13
Alopecurus agrestis	5,173	5,354	11,770	6,018	100	103	228	116
Atriplex patula	317	92	6	7	100	29	2	2
Caucalis arvensis	225	51	38	1	100	23	17	—
Euphorbia exigua	118	11	27	19	100	6	14	10
Galium aparine	210	44	50	54	100	21	24	26
Myosotis arvensis	788	620	295	187	100	79	37	24
Papaver spp.	29,227	16,245	7,311	2,109	100	56	25	7
Polygonum aviculare	726	47	5	47	100	6	1	6
Senecio vulgaris	231	81	10	11	100	35	4	4
Scandix pecten-veneris	260	46	142	115	100	18	55	44
Veronica buxbaumii	187	190	71	22	100	102	38	12
Group B								
Medicago lupulina	126	199	1,199	679	100	158	952	539
Stellaria media	182	673	1,193	488	100	370	655	268
Group C								
Alchemilla arvensis	6,664	9,303	9,540	4,518	100	140	143	68
Arenaria serpyllifolia	297	1,114	663	301	100	375	223	101
Bartsia odontites	216	397	196	312	100	184	91	144
Capsella bursa-pastoris	305	660	408	289	100	216	134	95
Legousia hybrida	798	694	1,078	701	100	87	135	88
Veronica hederaefolia	774	543	838	593	100	70	108	77
Veronica arvensis	2,760	7,170	3,777	921	100	260	137	33
Group D								
Anagallis arvensis	4	1	1	—				
Galium tricorne	42	6	2	2				
Grasses, chiefly *Poa annua*	51	304	61	51	Too few for percentages to have any			
Matricaria inodora	71	130	21	44	significance			
Sonchus arvensis	28	5	11	7				
Polygonum convolvulus	15	1	—	3				
Total of viable seeds	49,936	44,012	38,726	17,517	100	88	77	35

Since fallowing began in the autumn of 1925 the samples taken from each plot represent various stages of treatment, as one or more sections will have been fallowed while the others range from 1 to 4 years after their previous fallowing. As this occurs every year, with the different stages of treatment shifting from section to section, the changes in the weed-seed population indicated by the samples give a true idea of the cumulative result of the fallowing, as shown by the low figure reached by 1940. The numerical results are fully borne out by the notes made on the field at various times of the year.

indicates that any seasonal fluctuation may have been counterbalanced. The rise in 1930 occurred in the last year that the whole field was under crop. Henceforward, true seasonal fluctuations were again manifest, as they were now offset by the regular fallowing of one section per year. The years 1934 and 1938 were very clean and 1935–7 correspondingly weedy, but in spite of big differences in the annual population the tendency has been for a steady reduction. If the figures are considered at 5-yearly intervals (Table 12), the soil population decreased markedly after the initial long-term periods of fallowing and showed a continued reduction after the cycle of one

year's fallow in five was instituted. The fairest estimate of the decrease is to compare the weed-seed population in 1930, when all the field was cropped, with the average number per year for each 5-year period, during which each section had been fallowed for 1 year (Table 13).

TABLE 13. *Average weed-seed population per annum for three plots, 15 sq.ft.*

1930	44,125
1931–5	32,103
1936–40	26,770

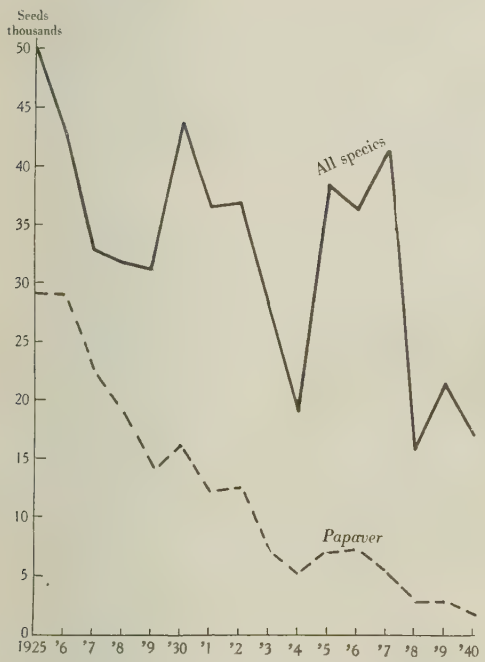


Fig. 5. Total number of weed seeds per year, 1925–40. Plots 2, 7, 18 (15 sq.ft.).

The steady reduction in weed seeds over the 10 years seems fully to justify the periodic 1-year fallow.

The prevalent weeds can be grouped according to their response to fallow and to their behaviour in succeeding years. These groups are set out in Table 12, the percentage figures giving the clearest picture of the trend of events.

Group A. *Reduction in numbers maintained*

All species in this group were reduced by the initial fallowing between 1925 and 1929 and have never again reached their original level. *Papaver rhoeas*, *Myosotis arvensis* and *Caucalis arvensis* steadily decreased from the beginning, only one specimen of *Caucalis arvensis* appearing in the 1940 samples. The reduction of *Papaver rhoeas* to 7 % affords the most spectacular change on the field, owing to the enormous number that used to occur. From the economic point of view this is probably the most important result of the whole fallowing scheme, as the abnormal amount of this strong-growing, broad-leaved weed had become a real menace to the wheat crop. *Atriplex patula*, *Senecio vulgaris* and *Aethusa cynapium* reached their lowest level by 1935, and then the two former remained stationary. *Polygonum aviculare*, *Euphorbia exigua*, *Galium aparine* and *Scandix pecten-veneris* were drastically reduced by 1930, the three latter showing a certain increase in the later years, probably only significant with *Scandix pecten-veneris*. The reduction in *Veronica buxbaumii* did not occur until the 5-year cycle of fallowing was begun, after which a rapid decline was noted.

The general behaviour of most of the species in this group is closely paralleled by what happened to them in the 3 years following the 1925–7 fallow (Brenchley & Warington, 1936, pp. 484–9). *Aethusa cynapium*, *Euphorbia exigua*, *Galium aparine* and *Papaver rhoeas* made little or no recovery, *Atriplex patula*, *Caucalis arvensis*, *Polygonum aviculare* and *Scandix pecten-veneris* remained practically stationary for 2 years with a certain increase later, the only species showing irregularity being *Myosotis arvensis*, *Senecio vulgaris* and *Veronica buxbaumii*. It therefore seems that if the response of the species in this group remains consistent, some of them may practically disappear, and others become quite insignificant, thus bringing about a definite change in the weed flora of the field, particularly as *Papaver rhoeas* is included.

Group B. *Increase in numbers maintained*

Two species only occur here, *Stellaria media* and *Medicago lupulina*, both of which are trailing plants with a long period of flowering, still at its height at the time of harvest. During the interval between cutting and ploughing, therefore, seed continues to ripen and restock the soil, and in a favourable season a big increase in soil population may easily occur. The spectacular rise in 1935 for both species may be associated with the fact that 1934 was a very clean year, heat and drought at critical periods hindering germination and checking weed development. This must have left a large supply of ungerminated seed lying dormant in the soil, in addition to the new seed produced in 1934, thus increasing the numbers available for the following season, which proved to be very favourable for both the species. *Medicago lupulina* is usually abundant on Broadbalk field on all the plots where any deficiency of nitrogen occurs, being scanty in the

presence of plenty of organic manure or inorganic nitrogen, so that in the three plots under detailed examination nearly all the *Medicago lupulina* records are from plot 18 with minerals and ammonium sulphate in alternate years. The behaviour of these species is consistent with that after the 1925–7 fallow, when both showed a rapid increase after fallowing, which in the case of *Stellaria media* was checked in the third year.

Group C. *Irregular increase and decrease in numbers*

Alopecurus agrestis is unique in its behaviour among the weeds occurring on Broadbalk. As its period of natural dormancy is short, a very large proportion of the seeds in the soil germinate and are cut down during the 1-year fallow period, reducing the surplus of viable seed to about 16–20 %. Most of this residue germinates with the crop the next year, and as seed production is prolific the numbers of seed in the soil reach or exceed their former level, usually within the year. Fallowing is therefore of no avail for the reduction of *Alopecurus agrestis*, but it has its value, nevertheless, as it checks the continued increase of the species and prevents it from becoming still more dominant. Under the present system of periodic fallowing there are signs of slight, steady increase which will need careful watching in case it assumes undue proportions.

Several of the species in this group germinate freely over long periods of the year, begin to flower and set fruit while still very small, continuing to restock the soil with ripe seed in this way throughout their lifetime. They may not always be reduced by fallowing, as if stubble ploughing is delayed, or if the periods between cultivations are unduly long, new plants may develop and fruit in the interval, thus working against the reduction caused by the fallow.

Capsella bursa-pastoris and *Arenaria serpyllifolia* were little affected by the earlier fallows, but afterwards responded to the increased soil fertility by a rapid increase in number, similar increases occurring with *Alchemilla arvensis*, *Veronica arvensis* and *V. hederaefolia* after their initial reduction by fallow. All these species, except the last, have declined from the high level reached in 1930, but it is not possible to attribute this definitely to the 5-year cycle of fallowing. Owing to their habit, a favourable combination of climatic conditions, time of ploughing and cultivation operations might easily result in another rise in any of these species. *Veronica hederaefolia* is typically a winter and early spring type, and its somewhat erratic behaviour is probably associated with the weather and cultivation conditions prevailing at that time each year.

Legousia hybrida and *Bartsia odontites* are very variable as regards their soil population. *Bartsia odontites* has already been shown (Brenchley & Warington, 1936, pp. 497–8) to be very irregular in germination and capable of lying dormant for long periods, and this is borne out by the more recent results. *Legousia hybrida* remains as much an enigma as ever, and it seems impossible to forecast what it will do either under crop or in fallow, its increase or decrease appearing to bear no relation to either form of treatment.

Group D. *Insufficient numbers to show trend of behaviour*

Where the number of buried weed seeds is very small, i.e. below 7–8 per sq.ft., it is unsafe to be too specific on the behaviour of the species from year to year, or under fallowing conditions. It does look, however, as though the response of *Matricaria inodora* and certain grasses is erratic, as their figures show no regular sequence, rising and falling very considerably. *Galium tricorne*, *Polygonum convolvulus* and *Sonchus arvensis*, on the contrary, were all more plentiful in 1925, before any fallowing was done, than at any time afterwards, and by 1940 the first two were quite insignificant or almost absent, *Sonchus arvensis* alone being slightly less reduced.

The periodic fallowing of Broadbalk field is being continued, with satisfactory results. Although annual samples are no longer taken, it is hoped to examine the same three plots in detail at intervals of 5 years, in order to supplement the field observations with some quantitative data, and so keep in touch with the general trend of the weed-seed population.

REFERENCES

Brenchley, W. E. & Warington, K. (1930). The weed-seed population of arable soil. I. Numerical estimation of viable seeds and observations on their natural dormancy. *J. Ecol.* **18**, 235.

—— (1933). The weed-seed population of arable soil. II. Influence of crop, soil, and methods of cultivation upon the relative abundance of viable seeds. *J. Ecol.* **21**, 103.

Brenchley, W. E. & Warington, K. (1936). The weed-seed population of arable soil. III. The re-establishment of weed species after reduction by fallowing. *J. Ecol.* **24**, 479.

Warington, K. (1936). The effect of constant and fluctuating temperature on the weed seeds in arable soil. *J. Ecol.* **24**, 185.

(*Received* 28 *March* 1945)

The effect of eyespot (*Cercosporella herpotrichoides* Fron.) on wheat and the influence of nitrogen on the disease

By Mary D. Glynne, W. M. Dion and J. W. Weil
Rothamsted Experimental Station, Harpenden, Herts

(With Plate 9 and 6 Text-figures)

The effect of eyespot throughout the season on wheat receiving different amounts of nitrogen was studied in pot experiments. Plants inoculated in December showed chlorosis of outer leaves in February. Among plants with high nitrogen, eyespot killed 11 %, caused straggling of 31 % and whiteheads in 14 % of the surviving ear-bearing straws, reduced yield of straw by 8 % and of grain by 16 %. The loss in straw yield was due to reduction in plant number, that of grain was about half due to reduction in number of ears and half to production of lighter grains. Among plants with low nitrogen the disease killed 23 % of the plants, caused straggling of 86 % and whiteheads in 18 % of the surviving ear-bearing straws, and reduced yield of straw by 23 % and of grain by 44 %. The loss in straw yield was due to death of plants, that of grain was about two-thirds due to the production of fewer ears and one-third to that of lighter grains. In the high-nitrogen series the number of shoots at the time of maximum tillering was reduced by 29 %; in the low-nitrogen series the disease caused reduction in height, a very uneven crop, delay in ear and anther emergence, and an increase in tail corn from 4 % in the controls to 30 % in the inoculated plants.

All inoculated plants became infected, but those receiving high nitrogen had only 49 % of the ear-bearing straws with severe lesions at harvest, while those receiving low nitrogen had 86 %. The larger number of tillers produced when nitrogen was applied may have enabled the less severely diseased shoots to survive and bear ears while the most severely infected died.

Introduction

Field observations and surveys show that eyespot, caused by *Cercosporella herpotrichoides* Fron., considerably reduces the yield of wheat, and also give information on the effects of rotation and other treatments on the incidence of the disease. They do not show how eyespot causes loss or the degree of loss which occurs under different conditions. An experiment was therefore set up in pots, so that detailed observations could be made throughout the season to determine the effect of eyespot on individual plants with a high- and low-nitrogen supply.

Methods

A poor soil, from a field in which cereals had not been grown for many years, was sieved and put into twenty glazed pots, 11 in. high and 10 in. diameter.

In a previous experiment when pots were close together eyespot spread from inoculated to control pots. To reduce the probability of spread the pots were filled only to within 3 in. of the top, so that the rim afforded some protection, and inoculated pots were separated from the controls by 6 ft. They remained out of doors in a bird-proof cage throughout the experiment.

Red Standard wheat seed was sown on 28 Nov. 1942, twenty-four grains in each pot. The plants were reduced to twenty per pot on 18 Dec., when they were about 1½ in. high: a few which germinated later were removed on 26 Feb. A control block of ten pots was not inoculated. Each plant in the other block was inoculated on 18 Dec. and again on 14 Jan. by placing at its base a piece (6 mm. diam.) of mycelial culture of the fungus grown on potato-dextrose agar. In each block five pots in random positions received ammonium sulphate equivalent to 3 cwt./acre on 3 Mar. Later, the plants which had received no nitrogen looked so poorly nourished that a basal dressing of ammonium sulphate, equivalent to 1 cwt./acre, was applied to all pots on 1 June. Each pot was given 250 c.c. water when the soil looked dry, to prevent loss of infection which sometimes occurs in dry spring weather when infected outer leaves wither before the fungus has penetrated to deeper layers.

Early yellowing and death of outer leaves

By the end of February the infected plants looked yellow and untidy, because the fungus caused decay at or near the bases of the leaf blades: the uninoculated plants were fresh and green. Ten weeks after the first inoculation there was some chlorosis in the first leaves of 72 % of the inoculated plants and in little more than 1 % of the uninfected controls. Later in the season the outer leaves of both healthy and infected plants turned yellow during normal senescence (Table 1).

TABLE 1. *Premature yellowing of young leaves*

(Plants in excess of twenty per pot appearing after 18 Dec. through late germination, were included in the counts made on 26 Feb. and then removed.)

Condition of 1st leaf	No. of plants 26 Feb. Control	Inoculated
Whole leaf green	206	57
Base of leaf yellow	3	96
Whole leaf yellow	0	49

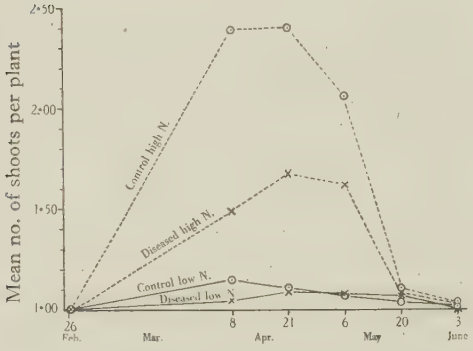
Death of plants

Surviving plants were counted at fortnightly intervals from 8 Apr. until harvest on 28 July. Three out of 200 control plants died and thirty-seven of the 200 inoculated plants. Eyespot increased the death-rate of plants with high nitrogen by 11 % and of those with low nitrogen by 23 % as compared with controls (Table 2).

Figures obtained in field surveys suggest that during the normal death of tillers occurring in spring, the more severely diseased tend to die, leaving healthier ones to produce ears. Plants with only one severely diseased shoot are more likely to die or to produce a severely infected shoot than are plants with several tillers some of which are less severely infected. This may explain the observation sometimes made in the field, that percentage infection seems to decrease at about the time when tillers die. This view is supported by the results of the present experiment. By harvest, in the low-nitrogen series, twenty-four plants had died and 86 % of the surviving ear-bearing straws had severe lesions, while among those with high nitrogen only thirteen plants had died and, of the survivors, 49 % had severe lesions. A preliminary experiment had given a similar result, 85 % of the plants in the low and 55 % in the high-nitrogen series showing severe lesions at harvest.

TABLE 2. *Number of plants surviving at different dates*

Treatment		Number of plants surviving								
Nitrogen	Eyespot	26 Feb.	8 Apr.	21 Apr.	6 May	20 May	3 June	17 June	1 July	28 July
High	Contr.	100	100	100	100	99	99	98	98	98
High	Inoc.	100	95	94	92	91	91	89	87	87
Low	Contr.	100	100	100	100	100	100	100	99	99
Low	Inoc.	100	96	89	89	83	81	78	78	76



Text-fig. 1. Mean number of shoots per plant.

Number of tillers

At the time of maximum tillering in April, plants with high nitrogen had an average of 2·4 shoots per plant in the control and 1·7 in the inoculated blocks, so that eyespot had reduced the number of shoots produced per plant by 29 %. Then tillers began to die until at harvest there was rarely more than one shoot per plant (Text-fig. 1). In the low-nitrogen series there were very few plants with more than one shoot at any time either among the control or the infected plants.

Height of plants

Measurements of the tallest plant in each pot, made at fortnightly intervals from 8 Apr. to 20 May, showed a tendency for diseased plants to be slightly but consistently shorter than controls. Measurements of every plant were then made on each of five dates from 3 June till harvest on 28 July.

Table 3 shows that lack of nitrogen reduced the height of healthy plants in June by about 8 in., but at harvest by only 2 in., and of diseased plants in June by 10 in. and 7 in. at harvest. Eyespot reduced the height of plants with high nitrogen in June by 2 in. and inappreciably at harvest, and of those with low nitrogen by 4–5 in. during the whole period from early June till harvest. The most rapid growth occurred between 3 and 17 June.

Height distribution curves

Distribution curves for height of plants made on five dates from early June to harvest showed progressive stages between those shown in Text-figs. 2 and 3. Control plants, both with high and low nitrogen, had fairly even crops with the greatest number of plants about the same height. Infected plants with high nitrogen had less steeply peaked curves, indicating a more uneven crop. At harvest there were a few more plants over 30 in. in the

inoculated than in the control pots with high nitrogen. This might indicate that the less severely diseased plants, fortified by nitrogen, were able to make bigger increases in height as a result of reduced competition, following early death of some of the most severely diseased plants and the failure of others to take up the available nitrogen. In early June most of the infected plants with low nitrogen varied from 6 to 15 in. They grew unequally, the majority at harvest ranging in height from 11 to 30 in., indicating that the disease inhibited growth much more in some plants than in others.

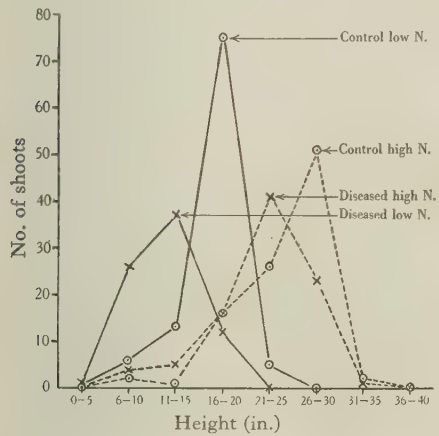
nitrogen, ear emergence took much longer and was spread over about 3 weeks.

In the high-nitrogen series anthers began to show about a week after ears had begun to emerge and, in the low-nitrogen series, after about 10 days. They gave curves closely resembling those obtained for ear emergence.

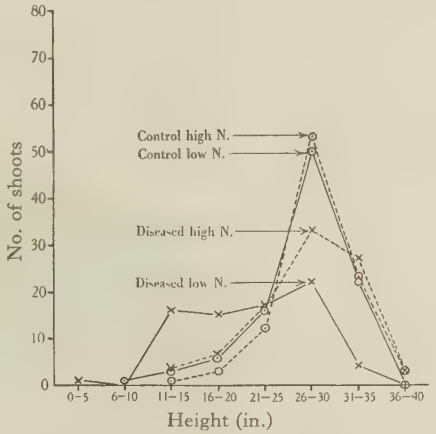
Thus, nitrogen deficiency delayed ear and anther emergence of uninoculated plants; infection by eye-spot had a negligible effect on plants with high

TABLE 3. *Heights of plants*

Treatment		Height: Of tallest plant (mean for 5 pots) (in.)				Height: Mean of all plants (in.)					Reduction in mean ht.
Nitrogen	Eyespot	8 Apr.	21 Apr.	6 May	20 May	3 June	17 June	24 June	1 July	28 July	in.
High	Contr.	11·6	16·6	19·0	25·1	24·6	27·3	28·1	27·8	28·6	
High	Inoc.	9·8	14·9	18·5	23·4	22·5	26·4	28·0	27·7	28·2	0·4
Low	Contr.	8·5	10·8	11·9	15·7	16·8	22·7	25·5	26·4	26·8	
Low	Inoc.	7·3	9·5	11·4	13·9	12·2	19·0	20·5	21·7	21·5	5·3



Text-fig. 2. Distribution of heights, 3 June.

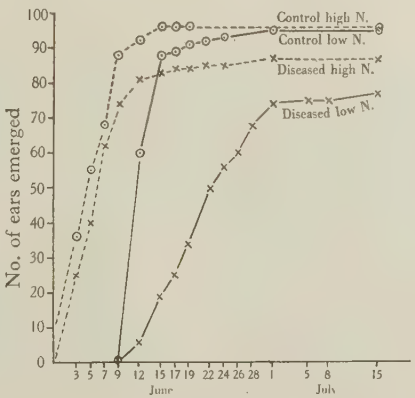


Text-fig. 3. Distribution of heights, 28 July.

Emergence of ears and anthers

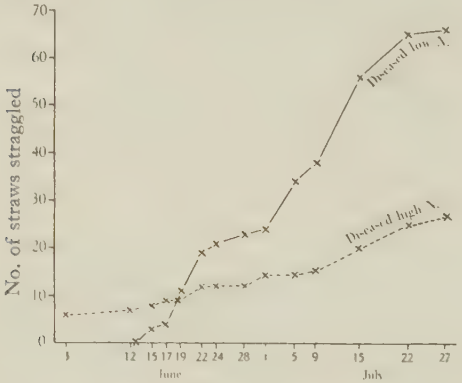
In the high-nitrogen series ears began to emerge in early June and nearly all were visible after 15 days. There was little difference between the date or rate of ear emergence of control and inoculated plants with high nitrogen, so that, allowing for early death of 11 % more infected than control plants, the two curves shown in Text-fig. 4 are very similar.

In the low-nitrogen series, ears began to show about a week later than in the high-nitrogen series. In the control block nearly all had emerged within 6 days, less than half the time taken by plants with high nitrogen. Among infected plants with low



Text-fig. 4. Date of ear emergence.

nitrogen, but on those with low nitrogen it considerably delayed and increased the time taken for emergence.



Text-fig. 5. Date of straggling.

Although straggling began at different dates in the high- and low-nitrogen series, it began in each at about the same time as the ears emerged, and so seemed to depend more on the state of development of the plants than on immediate external conditions.

Straws straggled at different dates were marked so that date of straggling could be related to other characters determined at harvest. Table 4 shows that severe lesions were found on all straws straggled by 28 June, on 50 out of 57 others straggled in July, but on only 23 out of 70 which remained upright. Thus, 92 % of the straggled straws and only 33 % of the upright ones had severe lesions; and, of the straws which had severe lesions, 58 % with high and 90 % with low nitrogen were straggled. This close association of severe lesions with straggling seems to indicate that depth of lesion was the main factor causing straggling, but that nitrogen enabled some severely diseased straws to remain upright.

Many of the shortest straws were straggled while others twice their height remained standing. Table 4 shows that straws which straggled in July were about the same height and gave yields similar to those which remained upright, but those which straggled in June were shorter and yielded only 51 and 67 % (in the high- and low-nitrogen series respectively) as much grain as the others. There was little difference in the numbers of grains per ear of straggled and upright plants.

Among upright straws with high nitrogen the severely diseased were found to be significantly shorter than the slightly or moderately diseased.

TABLE 4. *Date of straggling in relation to severity of eyespot, height of straw and yield of grain*

			No. of straws with eyespot			Mean ht. of straw in.		Yield of grain: Mean wt. per ear g.		Yield of grain: Mean no. per ear	
Nitrogen	Group	Date straggled	Slight	Moderate	Severe						
High	1	28 June	0	0	12	24·8		0·46		20·0	
	2	15 July	0	0	6	30·1		0·84		20·0	
	3	28 July	2	0	7	29·7	28·6	1·09	0·90	22·9	21·0
	4	Not straggled	9	33	18	28·2		0·88		20·8	
Low	1	28 June	0	0	18	19·5		0·31		14·1	
	2	15 July	1	3	26	22·5		0·48		16·6	
	3	28 July	0	1	11	23·1	22·6	0·46	0·46	14·7	15·4
	4	Not straggled	3	2	5	23·0		0·46		13·0	
High and low	1–3	Total straggled by 28 July	3	4	80	23·6		0·53		—	
	4	Upright at harvest	12	35	23	27·4		0·81		—	

Straggling

Poor crops, even when heavily infected, do not lodge, but individual straws fall down among the upright ones, resulting in an untidy condition known to farmers by dialect names, e.g. 'straggling' (Glynne, 1942). In this experiment the soil was not fertile enough to induce general lodging, but many infected straws straggled, while the uninoculated controls remained upright until harvest (Pl. 9, figs. 1, 2).

Diseased plants with high nitrogen began to straggle in early June (Text-fig. 5) and, by harvest, 31 % of the surviving ear-bearing straws had fallen. In the low-nitrogen series straggling began about 10 days later, and the number fallen increased until, by harvest, 86 % of the surviving ear-bearing straws were straggled.

Whiteheads

All the ears in the control pots ripened normally but, in the inoculated pots, white or prematurely ripened heads began to appear early in June. At harvest, 12 (14 %) and 13 (18 %) of the ear-bearing

straws had whiteheads in the high and low nitrogen series respectively.

Yield of straw and grain

Table 5 shows that infected plants yielded 8 and 23 % less straw than the controls in the high- and low-nitrogen pots respectively; a loss accounted for by death of diseased plants with no reduction in the yield of straw per plant.

Infection caused a loss of grain yield of 16 % in the high-nitrogen series. About half this loss was due to death of plants and the formation of fewer

(1) Slight; lesions superficial.

(2) Moderate; lesions dark, spreading round and beginning to penetrate the straw.

(3) Severe; lesions black, penetrating deeply into the straw base.

No plant in the uninoculated control block showed any sign of eyespot, whereas every plant in the inoculated block had an eyespot lesion. In Table 6 the numbers of grain-bearing ears are grouped according to the severity of infection, and the mean yield per ear recorded for each group. Plants with

TABLE 5. *Yield of straw and grain*

Treatment		Yield from 5 pots, wt. (g.)		No.				Wt. (g)			
Nitrogen	Eyespot	Straw error ±4·5	Grain error ±3·0	Grains	Plants	Ear-bearing straws	Grain-bearing ears	Straw per plant	Grain per ear*	1000 corn wt. (g.)	No. grains per ear*
High	Contr.	129	87·3	1981	98	95	95	1·32	0·92	44·1	20·9
	Inoc.	119	73·0	1808	87	88	87	1·37	0·84	40·4	20·8
Low	Contr.	78	52·8	1510	99	98	97	0·79	0·54	35·0	15·6
	Inoc.	60	29·7	1066	76	74	70	0·79	0·42	27·9	15·2
				Effect of eyespot							
High	Actual loss	10	14·3	173	11	7	8	−0·05	0·08	3·7	0·1
Low		18	23·1	444	23	24	27	nil	0·12	7·1	0·4
High	% loss	8	16	9	11	7	8	−4	9	8	nil
Low		23	44	29	23	25	28	nil	22	20	3

* In grain-bearing ears.

TABLE 6. *Severity of eyespot and yield of grain in fertile ears*

Treatment		Severity of eyespot infection							
		No. o		Slight		Moderate		Severe	
Nitrogen	Eyespot	No. of ears	Wt. grain per ear g.	No. of ears	Wt. grain per ear g.	No. of ears	Wt. grain per ear g.	No. of ears	Wt. grain per ear g.
High	Healthy	95	0·92	0	—	0	—	0	—
	Infected	0	—	11	1·20	33	0·96	43	0·66
Low	Healthy	97	0·54	0	—	0	—	0	—
	Infected	0	—	4	0·66	6	0·67	60	0·38

grain-bearing ears and half to the production of lighter grains. In the low-nitrogen series, infection caused a loss of 44 % in grain yield: 28 % was accounted for by the reduction in the number of grain-bearing ears and the remaining loss to the formation of lighter grains. There was little difference in the number of grains per ear of infected and control plants, but those with low nitrogen, both inoculated and control, produced only about three-quarters as many grains as did those with high nitrogen.

Severity of infection in relation to yield of grain

At harvest the plants were pulled up and diseased straws grouped according to the depth of eyespot lesion as follows:

only slight or moderate infection, at both levels of nitrogen, gave yields rather above the means obtained for control plants. This might be the result of compensation following reduced competition because of the early death of some of the diseased plants, as already suggested by height measurements, or it may indicate a selective action of the disease, the stronger plants showing least infection. In the latter case the comparison would be between the best of the plants in the inoculated block with the average in the controls. It seems likely that both factors may play a part.

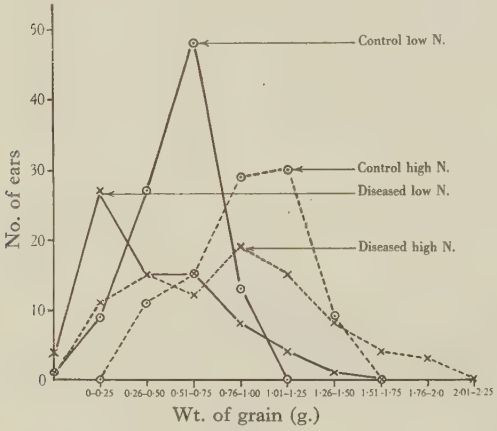
Severely diseased straws gave grain yields showing a loss of 28 and 30 % in the high- and low-nitrogen series respectively as compared with uninoculated plants.

Weight of grain produced per ear

Distribution curves for weight of grain produced by each ear (Text-fig. 6) show that diseased plants gave much flatter curves, indicating much less uniformity in yield among diseased than among control plants.

Among diseased plants, both with high and low nitrogen, some ears yielded a greater weight of grain than did any of the corresponding controls. The numbers are small, but support the suggestion that the less severely diseased among the inoculated plants had an advantage in competition for food as a result of early death and lowered food intake of the more severely diseased plants.

The numbers of grains per ear gave somewhat similar distribution curves. There was some evidence that in the low-nitrogen series severe infection



Text-fig. 6. Distribution of wt. of grain per ear.

resulted in fewer grains per ear than in the moderately and slightly diseased ears, but that the larger number of grains in the latter as compared with the mean number in the controls, compensated to an extent which gave mean values for the number of grains per ear which were nearly the same in infected and control plants.

Percentage of tail corn

Tail corn was separated from dressed corn by means of a sieve with meshes 2·25 × 20 mm. Reasonably good samples with less than 5 % of tail corn were obtained from healthy plants with high and low nitrogen, and from diseased plants with high nitrogen which had not straggled by mid-July, but those which had straggled earlier had a very high proportion (27 %) of tail corn (Table 7). Diseased plants with low nitrogen all had a high percentage of tail corn increasing with the earliness of straggling, but even those which remained upright until harvest had as much as 12 %. A comparison of all the diseased series with all the controls shows that, with high nitrogen, diseased plants gave a fairly good sample of grain with only 4 % tail corn similar to that obtained from control plants with low nitrogen, whereas diseased plants with low nitrogen gave an exceedingly poor sample which included 30 % of tail corn. This reduction in quality of grain is, therefore, another factor to be considered in assessing the effective loss from disease.

TABLE 7. *Tail corn from control, infected and straggled plants*

Condition		Tail corn (%) Nitrogen: High		Low	
Control	Upright	1		4	
Infected	Upright	1		12	
,,	Straggled after 15 July	2	4	26	30
,,	Straggled before 15 July	27		48	

DISCUSSION

Although conditions in pots differ widely from those in the field, the close study of individual plants at short intervals which is possible in pots, confirmed many general impressions obtained from field observations and helped in their interpretation. Various symptoms associated with eyespot in the field have generally been assumed to be caused by the disease, some with certainty, others rather doubtfully. The present experiment has shown that infection by *Cercosporella herpotrichoides* Fron. caused chlorosis of the outer leaves in February followed by the death of many plants. Among the survivors it caused straggling, whiteheads, delay in flowering and in growth, reduction in final height and a more uneven crop, as well as reduction in yield of straw and a greater reduction in yield of grain. These adverse effects were all reduced, and some eliminated, by the application of nitrogen.

In attempting to relate these results to those occurring in the field, at least three important differences in conditions must be considered. The spacing of plants in the pots differed from that normally found in the field, and this, together with the restricted food supply in pots, would affect competition in a way which can only be determined by experiment. Artificial inoculation resulted in the infection of every plant at about the same time, a condition rarely found in the field where some plants nearly always escape infection. The effect of the disease in pots is thus likely to be greater than in the field. Regular watering of pots in dry weather encouraged the persistence of infection, which, under natural conditions, might have been partially sloughed off.

Yield of grain obtained in these pot experiments is comparable with that obtained in the field under moderate to very poor conditions. In the uninocu-

a *b*

Fig. 1

a *b* *c* *d*

Fig. 2

GLYNNE, DION AND WEIL—EFFECT OF EYESPOT ON WHEAT AND INFLUENCE OF NITROGEN SUPPLY

lated high-nitrogen series it amounted to 27 cwt./acre, and in the low-nitrogen inoculated pots to only about 9 cwt./acre. The quality of grain, as shown by the percentage of tail corn, was similar to that obtained from moderate crops in the field except in the inoculated low-nitrogen series in which the percentage of tail corn was extremely high, resulting in a loss of dressed corn considerably greater than that estimated from total weight (see Table 5).

The effect of nitrogenous fertilizers on foot-rotting diseases has frequently been recorded in the field. In many instances confusion arises from the fact that no clear distinction was made between different foot-rotting organisms. Only those which refer specifically to eyespot will be considered here. Sprague & Fellows (1934) recorded that autumn applications of fertilizers rich in nitrogen increased eyespot. Hoffman (1934), in Germany, stated that eyespot is much more likely to flourish on rich than on poor soils. Weigert & Weizel (1935) stated that the disease was greater on plants with high nitrogen than on nitrogen-deficient plants. On Broadbalk field at the Rothamsted Experimental Station the disease is generally more abundant at harvest on the plots receiving nitrogen than on the poorly nourished plots. On the other hand, Oort (1936), in Holland, found in sand culture that the development of eyespot was favoured by nitrogen deficiency; and Meijers (1937), in field plots, found no appreciable difference in the percentage of straws infected at harvest, but that the loss attributable to the disease decreased with increasing applications of nitrogen.

Such apparently discrepant field observations are likely to result from the operation of two factors working in opposite directions, sometimes one and sometimes the other predominating according to conditions. In the field the application of nitrogen has two opposing effects on eyespot. The more luxuriant crops maintain a damper atmosphere near the soil level, and this favours the spread and development of the disease. This factor was eliminated in the pot experiments by frequent watering. On the other hand, the increased tiller production resulting from the application of nitrogen appears to enable the plant to discard the most severely diseased tillers and so reduce the intensity of infection at harvest. By this means, nitrogen reduces the adverse effects of the disease on the individual plants. As poorly nourished plants have a higher death-rate than those with high nitrogen, the percentage infection at harvest gives too low an estimate of the activity of the disease. These divergent effects of nitrogen are likely to produce different effects at harvest according to climatic conditions and to the date and rate of nitrogen application; both these factors need closer study.

The available evidence from field records and from pot experiments indicates that the effect of nitrogen in increasing yield and in reducing the damage done by eyespot considerably outweighs its ill effects in increasing the spread of the disease. Its application to infected crops appears to be especially desirable, so long as general lodging of the crop is not induced; the probability of lodging, as distinct from straggling, can be greatly reduced by using short, strong-strawed varieties.

REFERENCES

GLYNNE, MARY D. (1942). *Cercosporella herpotrichoides* Fron., causing eyespot of wheat in Great Britain. *Ann. appl. Biol.* **29**, 254–64.

HOFFMAN (1934). Bekämpfung der Fusskrankheiten des Weizens. (Control of the footrots of wheat.) *Dtsch. landw. Pr.* **61**, 570, 582.

MEIJERS, P. G. (1937). Een onderzoek naar factoren die van invloed zijn bij het optreden van de oogvlekkenziekte van de tarwe 1935. (An enquiry into factors that influence the occurrence of eyespot in wheat.) *Versl. RijkslandbProefs. 's Grav.* **43** (8) A, 271–311 (Groningen).

OORT, A. J. P. (1936). De oogvlekkenziekte van de granen, veroorzaakt door *Cercosporella herpotrichoides* Fron. (The eyespot disease of cereals caused by *Cercosporella herpotrichoides* Fron.) *Tijdschr. PlZiekt.* **42***e*, July–August, 179–234.

SPRAGUE, RODERICK & FELLOWS, HURLEY (1934). *Cercosporella* footrot of winter cereals. *Tech. Bull. U.S. Dep. Agric.* no. 428, pp. 1–24.

WEIGERT, J. & WEIZEL, H. (1935). Ertrags- und Güteminderung bei Getreide durch Fusskrankheiten. (Yield and quality depreciation in cereals through footrots.) *Prakt Bl. Pflanzenb.* **12**, 289–303.

EXPLANATION OF PLATE 9

Fig. 1. Wheat grown in pots in two blocks, (*a*) control, and (*b*) inoculated with *Cercosporella herpotrichoides* Fron. (The blocks were 6 ft. apart during the experiment but were moved nearer together for photographing.)

Fig. 2. High nitrogen: *a*, control; *b*, inoculated. Low nitrogen: *c*, control; *d*, inoculated.

A comparison of *Fusarium avenaceum* and *Fusarium caeruleum* as causes of wastage in stored potato tubers

By F. Joan Moore, Ph.D., *Rothamsted Experimental Station, Harpenden, Herts*

(With 1 Text-figure)

Fusarium avenaceum is reported for the first time as a cause of rotting of potato tubers in Britain. The progress of rotting in tubers infected with *F. avenaceum* has been compared with dry rot due to *F. caeruleum* in the laboratory, clamp and potato store. Of the four varieties, Majestic, King Edward, Doon Star and Arran Pilot, tested for susceptibility, King Edward was the most susceptible to *F. avenaceum* and Doon Star to *F. caeruleum*.

Optimum temperatures for growth on potato-dextrose agar were 20–25° C. for *F. avenaceum* and 20° C. for *F. caeruleum*; maximum temperatures were >30 and 30° C. respectively. For infection of wounded potato tubers, cardinal temperatures for *F. avenaceum* were similar to those on agar, but for *F. caeruleum* the optimum was 15° C. and the maximum 25° C. The optimum temperature for rotting tended, with both species, to be higher in the more susceptible potato varieties. At low temperatures *F. caeruleum* caused quicker rotting than did *F. avenaceum*, even though its rate of growth on agar was scarcely more than half that of the latter.

High humidity favoured rotting especially by *F. avenaceum*; *F. caeruleum* was more tolerant of relatively low humidity. Both species caused quicker rotting in the clamp than in store, even though there was no appreciable difference in mean temperature between the clamp and the store. This was attributed to the higher atmospheric humidity in the clamp.

Introduction

During 1943 many isolations of fungi were made by the writer from samples of rotting potato tubers collected by Drs A. R. Wilson and A. E. W. Boyd* in their survey of wastage in potato clamps. The principal cause of fungal wastage was blight due to *Phytophthora infestans*, the infected tubers becoming completely destroyed by the activities of saprophytic species of fungi and bacteria; next in importance was dry rot due to *Fusarium caeruleum* (Lib.) Sacc. A considerable proportion of the *Fusarium* rots was caused, however, not by *F. caeruleum* but by *F. avenaceum* (Fr.) Sacc., such rots being most frequently found in tubers of var. King Edward. *F. avenaceum* (*F. subulatum* App. & Wr.) has been reported as a wound parasite of potato tubers from the U.S.A. (Sherbakoff, 1915) and from Germany (Wollenweber, 1913; Appel & Fuchs, 1912; Schmidt, 1928) but not from Great Britain. For this reason and because of the frequency with which *F. avenaceum* had been isolated during the 1943 survey, the capacity of this species of *Fusarium* for rotting potato tubers was compared with that of *F. caeruleum*, a species already established as an important wound parasite in Great Britain and elsewhere (Sherbakoff, 1915; Schmidt, 1928; Pethybridge & Lafferty, 1917; Foister, 1940).

Experimental

In a preliminary trial *F. avenaceum* and *F. caeruleum* were found to infect wounded potato tubers but not uninjured tubers. A standard technique for the wounding and inoculation of tubers was therefore adopted in the experiments to be described below. A cylindrical wound approximately 6 mm. in diameter and 6 mm. in depth was made by stabbing the tuber with a metal rod, one end of which had been cut down on the lathe to the above dimensions. A 6 mm. disk from a colony of the *Fusarium* sp. on acidified potato-dextrose agar was placed in the wound as inoculum. Progress of the fungal rotting was determined at intervals during the course of an experiment by measuring the diameter of the superficially apparent lesion in two directions at right angles to one another; the average was recorded for each tuber. At the end of the experiment the inoculated tubers were cut open and the maximum depth of penetration of the rot was measured. A number of tubers were left uninoculated as controls, and none of these became infected by *Fusarium* spp.

Experiment I. Varietal susceptibility to infection. Tubers of four potato varieties, Majestic, King Edward, Doon Star and Arran Pilot, were inoculated with three different isolates of *F. avenaceum* and with three of *F. caeruleum*. Ten tubers of each variety were inoculated with each isolate, making sixty inoculated tubers of each variety; five tubers of each variety were left uninoculated as controls. The tubers were placed in boxes (lined with damp cotton wool and covered for the first month) which were placed on shelves in a brick potato store at the Midland Agricultural College. At the same time five tuber lots of the same varieties, which had been inoculated with the same six isolates and then kept

* Officers of the Agricultural Research Council stationed at the Midland Agricultural College.

in glass tumblers at 15° C. for 1 week, were also placed in similar boxes in the same store. Maximum and minimum temperatures were recorded daily in the store, the average temperatures for the three months being 4·5, 9 and 15° C. respectively. The diameter of the superficial rot was measured after 2 months and then again 1 month later, when the tubers were cut open and depth of penetration of the rot was also measured. Unfortunately, many of the tubers inoculated with *F. caeruleum* had decayed so far by this time that reliable average figures for all the isolates could not be obtained, and so figures for the depth of penetration of this species are not given. The results of this experiment are given in Tables 1 and 2.

In both series, i.e. with initial infection at 4·5 and 15° C. respectively, the tubers most susceptible to *F. avenaceum* were those of King Edward followed by those of Doon Star. Arran Pilot was least susceptible of the four varieties tested. In the series inoculated with *F. caeruleum* the most susceptible throughout was Doon Star. With the high initial temperature for infection Arran Pilot showed the least amount of rot. With low initial temperature for infection the relative susceptibilities of Arran Pilot and King Edward were reversed.

These results agree with the unpublished results of the clamp survey,* in which *F. avenaceum* was especially associated with rots of King Edward and *F. caeruleum* with those of Majestic. Furthermore, the results obtained with *F. caeruleum* confirm Foister's (1940) estimate of the relative susceptibility of these varieties under commercial conditions.

Experiment II. Effect of temperature upon the growth rate of F. avenaceum *and* F. caeruleum *on potato-dextrose agar.* Pilot cultures of three isolates of each *Fusarium* species were held at the five selected temperatures for 1 week. These five temperatures were obtained as follows: (1) in a cellar store at approximately 10° C., (2) in a controlled temperature store held at 15° C. and (3), (4), (5) in incubators held at 20, 25 and 30° C. From these pilot colonies disks of inoculum of 6 mm. diameter were taken for the experiment (except for the series of *F. caeruleum* plates at 30° C. which had to be inoculated from the pilot colony at 25° since, at 30° C., the fungus made scarcely any growth). Six plates of acidified potato-dextrose agar were inoculated with each fungus isolate for incubation at each of the five temperatures. Mean colony diameters at 1 week after inoculation of the plates for the six replicates of each series are shown in Fig. 1, isolates 104, 148 and 350 are *F. avenaceum* and 93, 127, 373 are *F. caeruleum*. The best temperature for growth was 20° C. for all three isolates of *F. caeruleum* and

TABLE 1. *Diameter of superficial rot (mm.)*

Variety ...	Majestic		King Edward		Arran Pilot		Doon Star	
	After 2 months	After 3 months	After 2 months	After 3 months	After 2 months	After 3 months	After 2 months	After 3 months
Inoculated and kept for 1 week at 15° C. before storage								
F. avenaceum	12 ± 3·1	23 ± 4·4	18 ± 3·8	> 40	2 ± 0·2	3 ± 0·2	18 ± 3·0	22 ± 4·6
F. caeruleum	36 ± 2·1	> 65	36 ± 1·6	> 50	30 ± 2·1	> 59	44 ± 1·9	> 76
Inoculated and put immediately into storage								
F. avenaceum	10 ± 2·2	17 ± 1·2	10 ± 1·1	29 ± 3·3	9 ± 1·4	9 ± 2·0	19 ± 1·7	22 ± 2·1
F. caeruleum	33 ± 1·2	> 70	17 ± 1·6	43 ± 3·0	27 ± 1·7	> 60	39 ± 0·8	> 75

TABLE 2. *Depth of penetration of rot for* F. avenaceum *only (mm.)*

	Majestic	King Edward	Arran Pilot	Doon Star
Inoculated and kept for 1 week at 15° C. before storage	18 ± 3·5	—	2 ± 0·5	16 ± 3·2
Inoculated and put immediately into storage	13 ± 2·9	17 ± 1·4	4 ± 1·2	20 ± 1·6

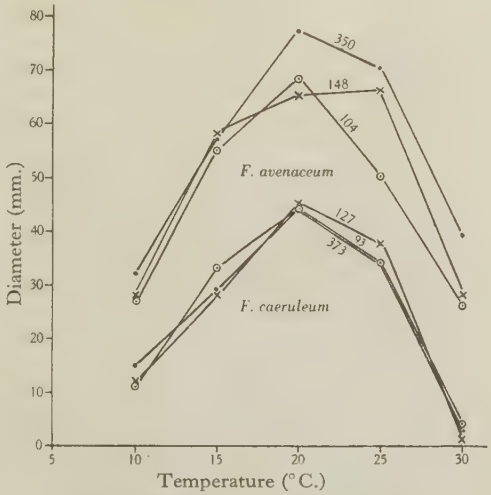


Fig. 1. Diameter of colony after 7 days.

* See introductory paragraph to this paper.

for two out of three isolates of *F. avenaceum*. The maximum for *F. caeruleum* was 30° C. or below, whereas for *F. avenaceum* it was above 30° C. At all temperatures the growth made by *F. caeruleum* was scarcely more than half that made by *F. avenaceum*. In general, the results suggest that *F. caeruleum* has a slightly lower optimum temperature for growth on agar than has *F. avenaceum*.

These results agree fairly well with those of other authors. Thus Moore (1924) found the optimum temperature for *F. caeruleum* to be 15–20° C. and the maximum 30° C.; Edson & Shapavolov (1920) give 25 and 30° C. for optimum and maximum temperatures. For *F. avenaceum*, De Haan (1937) reported an optimum of 24–27 and a maximum of 36° C., whereas Corneli (1934) found optimum and maximum temperatures for spore germination to be 20–26 and 34° C.

Figures from the two experiments were averaged and are given in Tables 3 and 4.

The results from this experiment show that the optimum temperature for potato rotting was 20–25° C. for *F. avenaceum* and around 15° C. for *F. caeruleum*. The maximum for *F. avenaceum* was above 30° C., and for *F. caeruleum* it was 25° C. or below. At the lower temperatures *F. caeruleum* caused more rapid superficial extension of the rot than did *F. avenaceum*, but only slightly greater penetration. Comparing these results with those obtained in Exp. II it will be seen that at low temperatures *F. caeruleum* spread more rapidly in the tuber than did *F. avenaceum*, whereas on agar *F. avenaceum* grew almost twice as fast. Moreover, although *F. caeruleum* was able to grow well on agar at 25° C., it did not produce a tuber rot at this temperature, and the optimum temperature for

TABLE 3. *Diameter of superficial rot (mm.)*

		Storage temperature (° C.)				
Fungus	Variety	10	15	20	25	30
F. avenaceum	Majestic	2±0·4	5±1·1	24±2·7	11±2·3	6±4·0
	King Edward	4±0·5	9±0·9	37±2·0	40±1·7	31±2·6
F. caeruleum	Majestic	8±0·3	17±1·4	15±2·1	1*	0
	King Edward	8±1·1	17±1·1	9±1·8	0	0

TABLE 4. *Depth of penetration of rot (mm.)*

		Storage temperature (° C.)				
Fungus	Variety	10	15	20	25	30
F. avenaceum	Majestic	1±0·3	3±0·7	11±1·9	5±1·8	2±2·1
	King Edward	4±0·6	7±0·7	17±1·3	17±1·4	10±1·7
F. caeruleum	Majestic	3±0·7	9±0·9	8±2·0	1*	0
	King Edward	3±0·6	10±0·8	3±1·4	0	0

* Only one tuber.

Experiment III. Effect of temperature upon infection. Majestic and King Edward potato tubers were inoculated with two isolates of *F. avenaceum* and with two isolates of *F. caeruleum*. Five tubers of each variety were inoculated with each fungus isolate, and three of each were left uninoculated as controls making forty-six tubers in all. Five such sets of forty-six tubers were placed in tall glass tumblers with lids and were stored at the five different temperatures obtained as in the previous experiment, viz. at 10, 15, 20, 25 and 30° C. The diameter of the superficial rot was measured after 16, 23 and 30 days, and the depth of penetration of the rot after 30 days. As the results of this experiment were of particular interest, the experiment was immediately repeated, again using two different isolates of each fungus, but with seven tubers in each series. In all other respects the methods used were the same as those in the preliminary experiment.

rotting was lower than for growth on agar. For *F. avenaceum* the cardinal temperatures were similar for both media. The results also show that *F. avenaceum* rotted tubers of King Edward more rapidly than those of Majestic, whereas, by contrast, *F. caeruleum* tended to rot tubers of Majestic more rapidly than those of King Edward. This result agrees with those obtained in Exp. I. It is of particular interest to note that the optimum temperature for rotting was higher when the fungus was in the more susceptible host variety, i.e. King Edward for *F. avenaceum* and in Majestic for *F. caeruleum*.

Experiment IV. Effect of atmospheric humidity upon infection. In this experiment one series of inoculated tubers was stored under conditions of relatively low humidity in a cardboard box and the other series was kept in covered glass tumblers with a layer of about 40 g. of wet sand in the bottom. The experiment was carried out at four different

temperatures: 10, 15, 20 and 25° C. Replicate lots of five tubers of Majestic and King Edward were inoculated with *F. avenaceum* and *F. caeruleum*, and three tubers of each variety were left uninoculated as controls. After 30 days the superficial rot was measured and then the tubers were cut and the depth of penetration of the rot was measured. The results are shown in Tables 5 and 6.

The effect of the high atmospheric humidity in increasing the external diameter of the rot (Table 5) is most pronounced in the series inoculated with *F. avenaceum*, in which the meagre infection at the effect of high humidity in increasing the depth of penetration of the rot is to be attributed more to its acceleration of initial infection than to any direct effect upon the susceptibility of the inner tissues of the tuber. Temperature, on the other hand, might well exert such a direct effect and its action affords an interesting contrast with that of humidity.

These results are very similar to those obtained by Goss (1921) in his experiments with *F. oxysporum*, *F. radicicola* and *F. tricothecioides*, in which he found that relative humidity played an important part in the amount of rot produced, the amount of rot

TABLE 5. *Diameter of superficial rot (mm.)*

Fungus	Variety	Humidity	Temperature (° C.) 10	15	20	25
F. avenaceum	Majestic	Low	3*	3 ± 0·9	38 ± 3·7	3 ± 2·0
		High	17 ± 4·9	14 ± 1·4	40 ± 6·4	> 50†
	King Edward	Low	3 ± 2·3	8 ± 1·0	44 ± 2·8	13 ± 3·6
		High	8 ± 1·9	13 ± 1·6	56 ± 1·7	> 74†
F. caeruleum	Majestic	Low	7 ± 2·0	18 ± 3·0	20 ± 2·7	‡
		High	13 ± 0·7	22 ± 1·7	25 ± 4·4	
	King Edward	Low	8 ± 1·7	23 ± 1·2	16 ± 3·2	
		High	14 ± 1·4	28 ± 1·0	11 ± 1·7	

* Only one tuber.

† Minimum values, two-fifths of the Majestic and four-fifths of the King Edward tubers having become completely rotten during the last month.

‡ These series omitted because of the negative results of previous experiments.

TABLE 6. *Depth of penetration of rot (mm.)*

Fungus	Variety	Humidity	Temperature (° C.) 10	15	20
F. avenaceum	Majestic	Low	4*	6 ± 2·0	15 ± 1·5
		High	7 ± 1·4	8 ± 1·1	15 ± 3·1
	King Edward	Low	4 ± 0·9	7 ± 1·0	10 ± 1·1
		High	5 ± 1·1	9 ± 0·4	17 ± 1·7
F. caeruleum	Majestic	Low	6 ± 2·2	9 ± 2·5	8 ± 1·0
		High	6 ± 0·7	8 ± 1·3	12 ± 1·0
	King Edward	Low	6 ± 1·4	9 ± 0·4	7 ± 2·5
		High	7 ± 1·3	10 ± 0·4	5 ± 2·0

* Only one tuber.

low temperature under dry conditions is also noticeable. In the series inoculated with *F. caeruleum* the differences are much less, but with one exception (King Edward at 20° C.) the superficial rot is greater in all the series held at high humidity.

Unfortunately, the humidity in the controlled temperature chamber at 20° C. was higher than that of the external atmosphere; this may explain the great amount of rot produced by both fungi in the low humidity series at this temperature. The depth of penetration of the rot (Table 6) was not significantly increased by high humidity except in King Edward inoculated with *F. avenaceum* at 20 and 25° C. and in Majestic inoculated with the same fungus at 25° C. It seems likely that this limited gradually increasing with increase in the relative humidity of the storage conditions. Weiss *et al.* (1928), in similar experiments, obtained a higher percentage of infection with *F. caeruleum* at the higher humidities, but depth of penetration of the rot was unaffected. Wollenweber (1913) stated that *F. avenaceum* behaved as a wound parasite only in conditions of high humidity. Schmidt (1928) found that a relative humidity of at least 80 % was necessary for infection by *F. avenaceum*, whereas *F. caeruleum* could infect tubers at a relative humidity of 50 %.

Experiment V. Effect of commercial storage conditions upon infection. In the experiments so far described the effects of the principal factors likely

to influence the progress of wastage of stored potatoes through attack by *F. caeruleum* and *F. avenaceum*, viz. varietal susceptibility, temperature and humidity of the atmosphere, have been investigated under controlled conditions. It now seemed desirable to see how far the experimental results could be used to explain possible differences in the progress of rotting caused by *F. avenaceum* and *F. caeruleum* under ordinary storage conditions, i.e. in potato clamps and stores. *Fusarium caeruleum* is well known as the common cause of dry rot under tray, bag and clamp storage conditions. *F. avenaceum* has so far not been encountered in tubers from trays or bags, but appears to be relatively common, at least in some varieties, in clamps.

In this experiment five King Edward tubers inoculated with *F. avenaceum* and five inoculated with *F. caeruleum* were placed in covered glass tumblers and kept at 15° C. for 1 week to ensure infection. Four tubers were uninoculated as controls. The tubers were then placed in a wire-netting bag, together with a similar number of tubers just inoculated and four uninoculated, and put into the end section of an experimental potato clamp at Rothamsted Experimental Station. At monthly intervals this end section, which consisted of about 15 cwt. of potatoes, was uncovered and progress of superficial rotting in the inoculated tubers was recorded. The tubers were then replaced in the clamp and the section was restrawed and earthed up again. Continuous temperature records were available for a similar section of the same clamp. A few days later other King Edward tubers from the same stock were similarly inoculated and placed in a brick potato store at the Midland Agricultural College. Results of this experiment are given in Tables 7 and 8.

Infection took place in most tubers under both conditions of inoculation and both conditions of storage. The amount of superficial rotting was slightly greater in the tubers held at 15° C. for 1 week after inoculation. In both series the effect of the storage conditions is striking, the tubers from the clamp showing considerably more superficial rot than those in the store. The effect is especially clear in the series inoculated with *F. avenaceum*. Differences in the depth of penetration of the rot under the different storage conditions are just significant in the tubers inoculated and held at 15° C. for 1 week but not in the others.

The differences in the amount of rot produced under the two storage conditions were obviously not due to differences in temperature, as temperatures were almost identical in store and clamp.

TABLE 7. *Diameter of superficial rot (mm.)*

	Clamp		Store	
	After 2 months	After 3 months	After 2 months	After 3 months
	Inoculated and kept at 15° C. for 1 week before storage			
F. avenaceum	24 ± 2·6	32 ± 3·4	8 ± 4·2	17 ± 7·5
F. caeruleum	35 ± 0·9	60 ± 1·4	25 ± 1·6	47 ± 3·0
	Inoculated and put immediately into storage			
F. avenaceum	17 ± 1·7	26 ± 3·1	7 ± 1·4	12 ± 2·4
F. caeruleum	26 ± 0·9	53 ± 0·9	10 ± 1·5	33 ± 5·0
Av. temp. (° C.)	6·5	14	8	15

TABLE 8. *Depth of penetration of rot after 3 months (mm.)*

Treatment	Fungus	Clamp	Store
Inoculated and kept at 15° C. for 1 week	*F. avenaceum*	19 ± 1·9	13 ± 1·4
	F. caeruleum	22 ± 1·2	15 ± 2·7
Inoculated and put immediately into storage	*F. avenaceum*	18 ± 1·5	13 ± 2·3
	F. caeruleum	20 ± 1·3	16 ± 3·3

Consideration of these results in conjunction with those from Exp. IV on the effect of humidity suggests strongly that the more rapid progress of the superficial rotting in the clamp than in the store was due to the higher atmospheric humidity in the former. If humidity was, indeed, the relevant factor determining this difference, then the much smaller difference in the depth of penetration of the rots in clamp and store could also be explained by reference to the results of Exp. IV. Another possibility remains, however, to be considered—that the progress of the rotting was favoured in the clamp by the accumulation of volatile excretions from the tubers. To test this possibility a further experiment was set up.

Experiment VI. Effect of humidity and forced aeration upon infection. Wide-mouthed 5 l. glass bottles plugged with cotton-wool were used as containers for the inoculated tubers in this experiment. The bottles were divided into four series of three

each. In the first series an open dish of concentrated sulphuric acid was placed in the bottom of each bottle to ensure low atmospheric humidity; in the second a similar dish of water was placed, to produce high atmospheric humidity; through the third was pumped a continuous current of moist air; the fourth was used as a control. Thus a series of storage chambers with different degrees of atmospheric humidity was obtained. The object of the forced aeration in the third series of bottles was to remove any volatile excretions of the inoculated tubers, but an incidental effect of this treatment was to increase the atmospheric humidity to such a high level that the surfaces of the tubers were actually wet at the end of the experiment and many had become completely rotten. Five tubers of each of the two varieties Majestic and King Edward were inoculated with *F. avenaceum* and five with *F. caeruleum*; four tubers (two of each variety) were left uninoculated and placed with the others in the jars as controls. Thus each jar contained twenty-four tubers. The jars were covered with black paper and left for 1 month in the laboratory, the average temperature of which during the period of the experiment was 19° C. After 1 month both the superficial rot and the depth of penetration of the rot were measured. The results are shown in Tables 9 and 10.

The results show a slight reduction, more marked in Majestic, in the extent of superficial rot with decreasing humidity. The depth of penetration of the rot was not significantly different in the different series except where the tubers had become completely rotten and had been invaded by bacteria. As the most rapid extension of the rot was found in the continuously aerated series, it seems unlikely that the susceptibility of the potato tuber to infection in the clamp is increased by the accumulation of volatile excretions.

These results confirm those of Weiss *et al.* (1928), who found that the amount of infection by *F. caeruleum* was independent of the rate of air exchange in a comparison between practically still air and a complete change four times an hour.

I wish to thank Dr A. R. Wilson for his advice and help in providing the tubers and facilities for the experiments carried out at the Midland Agricultural College, Mr S. D. Garrett for his helpful criticism and advice throughout the work, and also Dr W. L. Gordon for identifying the culture of *Fusarium avenaceum*. The work was carried out with the aid of a grant from the Agricultural Research Council.

TABLE 9. *Diameter of superficial rot (mm.)*

Fungus	Variety	H_2SO_4	Control	Water	Air pump
F. avenaceum	King Edward	$22 \pm 1{\cdot}6$	$24 \pm 2{\cdot}2$	$24 \pm 2{\cdot}1$	> 29
	Majestic	$13 \pm 2{\cdot}1$	$17 \pm 1{\cdot}8$	$19 \pm 1{\cdot}9$	$21 \pm 1{\cdot}8$
F. caeruleum	King Edward	$27 \pm 1{\cdot}8$	$28 \pm 1{\cdot}4$	$27 \pm 0{\cdot}8$	$33 \pm 1{\cdot}9$
	Majestic	$26 \pm 1{\cdot}5$	$28 \pm 1{\cdot}2$	$31 \pm 1{\cdot}1$	> 42

TABLE 10. *Depth of penetration of rot (mm.)*

Fungus	Variety	H_2SO_4	Control	Water	Air pump
F. avenaceum	King Edward	$5 \pm 1{\cdot}2$	$8 \pm 1{\cdot}8$	$6 \pm 1{\cdot}6$	> 30
	Majestic	$7 \pm 1{\cdot}1$	$6 \pm 1{\cdot}5$	$6 \pm 2{\cdot}7$	$7 \pm 1{\cdot}9$
F. caeruleum	King Edward	$9 \pm 1{\cdot}5$	$7 \pm 0{\cdot}9$	$9 \pm 0{\cdot}6$	$8 \pm 0{\cdot}4$
	Majestic	$7 \pm 1{\cdot}8$	$8 \pm 0{\cdot}8$	$7 \pm 1{\cdot}7$	> 30

REFERENCES

APPEL, O. & FUCHS, J. (1912). Zur Kenntnis der Fusariumfäule der Kartoffeln. *Mitt. biol. Anst.* (*Reichsanst.*), *Berl.*, **14**, 16.

CORNELI, E. (1934). Temperature di germinazione di spore fungine in relazione alle infezioni sugli ospiti. *Nuovo G. bot. ital.* **41**, 121.

DE HAAN, J. T. (1937). Untersuchungen über das Auftreten der Keimlings-Fusariose bei Gerste, Hafer, Mais und Reis. *Phytopath. Z.* **10**, 235.

EDSON, H. A. & SHAPAVOLOV, M. (1920). Temperature relations of certain potato rot and wilt-producing fungi. *J. agric. Res.* **18**, 511.

FOISTER, C. E. (1940). Dry rot diseases of potatoes. *Scot. J. Agric.* **23**, 63.

GOSS, W. (1921). Temperature and humidity studies of some Fusaria rots of the Irish potato. *J. agric. Res.* **22**, 65.

MOORE, E. S. (1924). The physiology of *Fusarium caeruleum*. *Ann. Bot., Lond.*, **38**, 137.

PETHYBRIDGE, G. H. & LAFFERTY, H. A. (1917). Further observations on the cause of dry rot of the potato tuber in the British Isles. *Sci. Proc. R. Dublin Soc.* **15**, 193.

SCHMIDT, E. (1928). Schädigungen der Kartoffel durch Pilze der Gattung *Fusarium* (Lk.). *Arb. biol. Abt.* (*Anst. Reichsanst.*), *Berl.*, **15**, 537.

SHERBAKOFF, C. D. (1915). Fusaria of potatoes. *Mem. Cornell agric. Exp. Sta.* no. 6.

WEISS, F., LAURITZEN, J. I. & BRIERLEY, P. (1928). Factors in the inception and development of *Fusarium* rot in stored potatoes. *Tech. Bull. U.S. Dep. Agric.* no. 62.

WOLLENWEBER, H. W. (1913). Studies on the *Fusarium* problem. *Phytopathology*, **3**, 44.

(*Received* 19 *April* 1945)

The effect of disinfecting and bruising seed potatoes on the incidence of dry rot (*Fusarium caeruleum* (Lib.) Sacc.)

By T. Small, Ph.D., A.R.C.S., *Agricultural Advisory Department, Manchester University*

The effect of bruising and of disinfecting ('dipping') seed potatoes with a proprietary organo-mercury preparation on the incidence of dry rot in them was tested in field trials during three seasons. The tubers used were of the susceptible variety Ninetyfold, taken from crops grown in contaminated soil, harvested immature in July to early August each year under farm conditions, and stored in boxes.

Seed tubers not deliberately bruised, whether dipped or not at lifting time, remained practically sound until planting time in the following season, if left undisturbed in their boxes.

Tubers deliberately bruised, either at digging time or 1–2 weeks later, but not dipped, developed severe dry rot with few exceptions. The disease had run its course by mid-October. When undipped, sound tubers were bruised in October, they contracted severe dry rot, but dipping such tubers immediately before bruising reduced the loss satisfactorily in five out of six trials.

Tubers bruised at digging time and immediately dipped suffered little from dry rot in almost all cases. Delayed dipping of bruised tubers checked the disease in some trials but not in others. Seed tubers severely bruised 1–2 weeks after being dipped remained practically sound except in one instance, whereas tubers severely bruised approximately 3 months after being dipped, subsequently developed severe dry rot in four out of six tests, unless they had been redipped immediately before they were bruised.

Inoculation of healthy tubers with soil samples showed that the fungus is widely distributed in potato fields in Cheshire. Dipping killed all, or almost all, of the fungus in the soil adhering to the seed tubers.

The results are discussed and suggestions are made for further investigations and for practical control measures.

Introduction

In a previous paper (Small, 1944) the writer showed that *Fusarium caeruleum* (Lib.) Sacc.,* which causes potato tuber dry rot, exists quite commonly in field soils in which potatoes have been grown and also in the soil adhering to the lifted tubers. The fungus remains in a viable condition on tubers reserved for seed until planting time in the following spring. Obviously, therefore, the disinfection of such surface-contaminated seed potatoes at an early stage might be expected to prevent dry rot, and already some preliminary work on these lines has been done. Foister (1940), in a general account of the disease in Scotland, recommended dipping seed tubers in a disinfectant at lifting time, while Foister & Wilson (1943) concluded that the only satisfactory time for such treatment was immediately after lifting. Full experimental details of these investigations have, however, not yet been published.

It has long been known that the dry-rot fungus is essentially a wound parasite, and that the incidence of the disease is closely associated with the amount of mechanical damage suffered by the tubers during lifting and subsequent handling. Consequently, in the present investigation attention has been paid not only to the disinfection of contaminated seed tubers, but also to the serious danger arising from mechanical damage to them both before and after treatment. The trials were made during the three years 1942–44 on ten farms in north Cheshire, that of the University Field Station at Warburton being one of them. The results obtained in 1943 and 1944 agreed so well with those of 1942 that, to save space, the latter have been excluded from the present account. The farms were the same in both years, though the crops were grown on different fields.

* This species of *Fusarium* is believed to be the one most commonly responsible for potato dry rot in this country, although other species have been known to cause a similar rot. The writer's investigations on dry rot, so far, have been mainly of the nature of field trials aiming at the development of some practical method for controlling it, and the question as to the specificity of the causative parasite, not being of immediate importance, has been left for future study.

Treatments

Growing and lifting the crops

Except on farm 1 where Arran Pilot was grown in 1944, the early variety Ninetyfold was used because it is very susceptible to dry rot and is usually lifted in the district concerned before blight (*Phytophthora infestans*) appears. The seed was planted between mid-March and mid-April, and the crops were lifted by the farmers and their own men under ordinary commercial conditions, which varied slightly from farm to farm, on dates varying from 6 to 27 July in 1943, and from 24 July to 8 Aug. in 1944, except at farm 3, where lifting took place on 22 June. With

few exceptions all the crops were harvested immature in fine weather and, except at farms 5 and 9 in 1943, with only a little soil adhering to the tubers. The 'spinner' was used for digging the crops in all the trials except those at Warburton, where the far less harmful potato plough was employed. From the tubers thrown out by the spinners the ware was removed by hand and the remaining seed was then gathered by hand into 1 cwt. hampers and afterwards tipped into seed boxes, each holding $\frac{1}{8}$ cwt.; on farm 1, in 1943, the tubers were (by mistake) collected into sacks instead of hampers. At Warburton the seed tubers were placed directly into seed boxes in the fields. No riddling was done with any of the crops. The seed in the boxes was used for the disinfecting and bruising tests, the boxes of seed being afterwards stored in barns or lofts.

Treatment of freshly dug seed tubers

(i) *Disinfection.* Preliminary laboratory experiments had shown that the proprietary organomercury fungicide 'Aretan' satisfactorily checked dry rot, and it was used throughout the trials except on farm 3 in 1944, where a 1 % dilution of 40 % formaldehyde was employed. Four trials showed that 1 % Aretan was not more effective than a 0·5 % solution, and it was therefore used at the latter concentration. The boxes of seed were immersed in a 15 gal. bath of this solution and moved to and fro for 1 min. to ensure thorough wetting of the tubers. After lifting out of the bath and draining off the excess liquid from the boxes, they were stored in lofts or barns until October when all were transferred to lofts for the winter. Before immersing a fresh box of seed in the bath, $\frac{1}{4}$ pint of 1 % Aretan solution was added to it to maintain the necessary strength of the fungicide. This process of tuber disinfection is referred to throughout the paper as 'dipping'.

(ii) *Bruising.* Apart from, and in addition to, a certain amount of accidental damage to the seed tubers, consequent on the mode of lifting and handling them, they were deliberately subjected in certain of the tests to further mechanical injury by bruising. The 'standard bruise' was obtained by tipping each box of seed into a 1 cwt. wicker hamper, which was then shaken vigorously thirty times. This rough and ready but practical method was almost always effective in producing additional extensive skin abrasions on freshly dug seed. When still more extensive damage was desired, in particular to simulate the worst that would be likely to occur during transport by rail or sea in sacks, the tubers (but not any residual soil in the boxes) were transferred by hand to sacks. These were then vigorously shaken and trampled on. After these rather drastic treatments, the seed tubers were returned to their original boxes and stored alongside the others. In this account the terms 'bruised' or 'not bruised' refer exclusively to the above additional maltreatment or its absence, and not to accidental skin abrasions or superficial scratches often present before treatment and resulting from the mode of digging and handling whilst harvesting the crop.

(iii) *Precautions against recontamination.* To avoid contamination from extraneous sources the following precautions were taken and were found to be adequate. All hampers used for bruising were soaked in Aretan solution, washed and dried; the sacks were steam sterilized. At bruising time the hampers and sacks were placed on linoleum which, together with the rubber boots worn when trampling on the sacks of tubers, was washed with Aretan. Boxes of dipped tubers were stacked above undipped ones and were examined first. Old, non-disinfected seed boxes were employed except at Warburton in both years, and at farms 5 and 6 in 1944 where new or disinfected boxes were used. In all the dipped series the seed was stored throughout the trials in boxes dipped at the same time as the seed.

(iv) *Details of experimental treatments.* The seed tubers were subjected to the seven series of dipping and bruising tests listed below; in each series three or more boxes of seed were used, and each box was treated separately. All the treatments were carried out on the farms by the writer assisted by the foreman of the University Field Station.

Series 1. Not bruised; not dipped.
Series 2. Not bruised; dipped at lifting time.
Series 3. Standard bruise at lifting time; not dipped.
Series 4. Standard bruise at lifting time; then dipped at once.
Series 5. Standard bruise at lifting time; then dipped after 7 days.
Series 6. Not bruised and not dipped at lifting time; severely bruised 14 days (in 1943) or 7 days (in 1944) later.
Series 7. Not bruised but dipped at lifting time; severely bruised 14 days (in 1943) or 7 days (in 1944) later.

RESULTS

Examination of the treated tubers and results of the treatment

The boxes in series 3 and 6 were examined in both years in September and again in mid-October when the final examination of all the series (1–7) was carried out. By this time practically all the dry rot that occurred had become visible, for, during the remainder of the storage period, amongst the tubers that remained undisturbed in their boxes, very little further attack developed. Some of the boxes of seed from which all diseased tubers had been removed were used for further trials in October, and the results of these will be given later. No blight

(*P. infestans*) appeared; the losses were due entirely to dry rot.

The results obtained in mid-October 1943 and 1944 are summarized in Table 1, in which the figures represent the total percentage loss of tubers (by number) for each series, not box by box. Losses below 1 % are entered as nil. The total numbers of tubers involved in all seven series at each farm in each year varied from about 2500 to over 7000, the average being 4732, so that the percentages given may be regarded as based on adequate numbers of tubers examined. Rather large-scale field trials, such as those described, are particularly subject to somewhat extensive experimental errors due to variation in condition of soil, crop rotation, manuring, handling at lifting time, degree of immaturity of the tubers, etc. Even differences in the results within a single series on each farm may be expected, although these occurred only where the losses were heavy and especially in series 3 and 6. The rather crude method of standard bruising may also have been accountable for some variation. Moreover, wide variations have also occurred in more carefully controlled laboratory tests. One factor that, perhaps, deserves attention is the variation in the degree of contamination from tuber to tuber. One special trial, in which the natural contamination of the tubers was reinforced by spraying them as uniformly as possible with a spore suspension of the fungus, showed that this increased amount of inoculum produced a distinct increase in the amount of disease, although the variation in percentage of attack remained practically the same from box to box. It is believed, however, that such discrepancies as arose during the course of the trials do not invalidate the main conclusions that can be drawn from the results, which are now to be discussed.

Effect of bruising at lifting time

In series 1 (tubers unbruised and not dipped) the losses were very low, exceeding 5 % in only two cases; that of 13 % at farm 1 was probably due to harvesting (by mistake) into sacks instead of hampers. The almost total absence of dry rot in both years at Warburton (farm 8) was probably due to lifting by plough instead of by spinner and direct transfer to boxes, avoiding hampers. This noteworthy result merits serious consideration. The results as a whole show that tubers of a susceptible variety, grown in contaminated soil, lifted immature with a spinner and tipped from hampers into boxes and allowed to remain undisturbed may not develop much dry rot.

In other tests at Warburton, in 1942 and 1943, freshly dug tubers were harvested (*a*) direct into boxes, (*b*) into hampers and tipped into boxes at once, (*c*) into sacks, carted to store and tipped into boxes at once. The losses were, (*a*) nil in both seasons, (*b*) 5 % (1942) and nil (1943), (*c*) 17 % (1942) and 12 % (1943). The harmful effect of harvesting into sacks is clear, and the great advantage of handling as little and as carefully as possible is also evident.

In series 3 (standard bruise as soon as dug, but not dipped) the losses were heavy, ranging from 20 to 51 % in ten trials, although almost all the crops were exceptionally clean when lifted. The relatively small losses at farms 1 (1944), 2 (1943), 6 (1944) and 7 (1944) are interesting, since at each place (except farm 2) the bruising treatment caused extensive skin abrasions, sufficient to give heavy loss on the other farms. The reason for this result is not apparent, but it would seem that bruising contaminated tubers does not invariably lead to severe dry rot. It may be noted, however, that serious dry rot did occur on three of these farms in series 6.

The general conclusion to be drawn from a comparison of series 1 with series 3 is that heavy bruising greatly accentuates the development of dry rot in seed tubers. No doubt infection comes from adhering soil. It was found in earlier work (Small, 1944) that the disease does not spread to any extent from diseased to healthy tubers stored in the same box. Here also, in series 3, after removal of the numerous affected tubers bearing external spore pustules in mid-October, those remaining undisturbed in the boxes developed little or no further disease during subsequent storage.

Effect of dipping at lifting time

In series 2 (unbruised, dipped as soon as dug) dry rot never exceeded 2 % and was less on every farm than in series 1. Comparison of the two series might suggest that dipping of unbruised tubers is superfluous, but this would hold good only if the tubers were allowed to remain undisturbed in their boxes until planting time. What might happen after further handling will be shown later. Incidentally, the negligible loss in series 2 suggests that little, if any, infection had occurred before lifting, although the field soils were contaminated.

In series 4, where the tubers were bruised at lifting time and then dipped at once, the disease was in general reduced, though not nearly so markedly, for some obscure reason, on farms 3 and 7 in 1943. Even apart from these exceptions, rather more dry rot appeared than in series 2, and this was no doubt due to bruising before dipping. The result may, perhaps, help to explain the 4–5 % losses in dipped tubers in trials made by Foister & Wilson (1943). It seems fair to conclude that immediate dipping after excessive bruising at lifting time does reduce very considerably the incidence of dry rot.

A dipping trial with formaldehyde (1 % dilution of 40 % formaldehyde) at farm 5 in 1944 (Table 1),

TABLE 1. *Effect of bruising and dipping Ninetyfold seed potatoes in Aretan on ten farms in Cheshire in* 1943 *and* 1944; *showing dry-rot percentages*

	No. of farm ...	1		2		3		4		5		6		7		8		9	10
Series	Year	1943*	1944†	1943	1944	1943	1944	1943	1944	1943	1944‡	1943	1944	1943	1944	1943	1944	1943	1943
1	Not bruised; not dipped	13	1	0	3	8	3	3	4	4	4	5	4	3	0	0	0	3	0
2	Not bruised; dipped at lifting time	2	0	0	0	1	1	1	0	2	0	0	0	1	0	0	0	1	0
3	Standard bruise at lifting time; not dipped§	32	0	5	14	51	34	17	22	30	35	20	7	33	5	21	13	32	10
4	Standard bruise at lifting time; then dipped at once	7	3	0	4	19	5	6	1	2	9	6	2	17	0	2	4	4	1
5	Standard bruise at lifting time; dipped 7 days later	—	—	—	18	—	—	—	—	—	—	—	8	—	5	—	0	—	—
6	As series 1, but severely bruised 1 or 2 weeks after lifting	17	50	50	42	54	25	40	65	59	47	51	47	42	6	27	28	25	43
7	As series 2, but severely bruised 1 or 2 weeks after lifting and dipping	6	3	0	3	9	2	7	1	21	1	1	3	2	0	0	0	1	3

* Crop harvested into sacks instead of hampers.
† Var. Arran Pilot.
‡ Dipped in 1% formaldehyde instead of in Aretan.
§ Skin abrasions were extensive in all tubers deliberately bruised at lifting time, except at farms 2 and 10 in 1943.

reduced dry rot from 35 to 9 %, but in a similar test at Warburton 28 % of the treated seed developed the disease; further experiments with this fungicide are needed before its practical value can be assessed.

Delayed dipping of bruised tubers

Dipping 7 days after lifting and bruising (series 5) was tried on only four farms, in 1944. It checked the disease on farm 8, but not on farm 2; on farms 6 and 7 the results were inconclusive because little dry rot occurred at these farms in series 3.

Further trials with freshly dug Ninetyfold were made at Warburton in July 1944. Six boxes containing 992 tubers in all were first contaminated by spraying each tuber with a spore suspension of the fungus, then standard bruised and afterwards stored in boxes. After 3 days, half the tubers were dipped; the loss after storage in boxes for 3 months was 43 % in the dipped and 39 % in the undipped. In another test, however, tubers bearing contaminated soil, but not sprayed with a spore suspension, were bruised at lifting time and dipped 7 days later. These remained sound in their boxes, whilst the bruised, undipped, control tubers suffered a loss of 13 %. In a third experiment the tubers were first inoculated with a spore suspension, then bruised, and afterwards stored for 14 days in sacks; they were then tipped into boxes and half of them were dipped. The loss in the dipped seed was 9 % and in the undipped 15 %.

These somewhat variable results at least suggest that it is risky to delay the dipping of bruised tubers, but further work on the matter is needed. From the practical point of view it seems very likely that commercial seed bruised in transit would ultimately develop much dry rot even if dipped by the farmer on receipt. Thus dipping of seed immediately on its arrival by farmers importing it by rail or sea, is unlikely to be a successful method of suppressing the disease. Foister & Wilson (1943) concluded that the only satisfactory time for dipping was immediately after lifting. This view is supported by the above results; but, as shown later, delayed dipping of unbruised tubers proved effective in five out of six tests.

Delayed bruising of dipped tubers

Unbruised tubers, either undipped (series 6) or dipped (series 7) at lifting time, were severely bruised in sacks 14 days (1943) and 7 days (1944) later. In series 6 the losses up to mid-October were very high, ranging from 40–65 % in twelve trials, from 17 to 37 % in five trials, and were low (6 %) in only one case (farm 7 in 1944). These heavy losses are in marked contrast to those in series 7 in which, with the exception of farm 5 in 1943, the highest loss was only 9 %. The result at farm 5 will be discussed later. The general result suggests that seed tubers, if dipped at lifting time, would successfully withstand the bruising incident to transport and remain relatively free from dry rot.

At almost all the farms the losses in series 6 were far greater than those in series 3, no doubt owing to the more severe bruising. In view of this the small loss of 6 % at farm 7 in series 6 in 1944 is interesting. Only 4 years elapsed here between successive potato crops; a standard potato fertilizer (½ ton/acre) but no dung was applied in 1944; and the haulms were partly green at lifting time. Bruising caused extensive skin abrasions on the immature tubers; the *p*H of the soil was 6·71, but in the course of this work the fungus has been found to be viable in five soils with *p*H values ranging from 6·26 to 6·74. These conditions are not exceptional and fail to explain the result. Inoculation with the soil sample collected in the field, however, gave almost negative results (see Table 2), but this is not sufficient proof that the whole field is uncontaminated. Additional soil samples are being tested.

TABLE 2. *Inoculation of healthy tubers with soil samples scraped from undipped and dipped tubers*

Year	Source of soil samples	Range of *p*H values	No. of farm and no. of tubers infected out of 18									
			1	2	3	4	5	6	7	8	9	10
1943	Undipped tubers (field samples)	5·25–5·85	12	18	15	15	11	18	17	—	10	10
1944	Do.	4·91–6·71	12	15	7	18	15	7	1	18	—	—
1943	Dipped tubers (series 7)	—	0	1	0	2	12	0	0	—	0	8
1944	Do.	—	0	0	0	0	2	0	0	0	—	—

Presence of the fungus in the soil adhering to the tubers

Two samples of the soil adhering to the tubers were collected at each farm both in 1943 and 1944. One was taken in the field immediately after lifting the crop and before the tubers could become contaminated from any extraneous source; the other from the tubers in series 7 after they had been dipped but just before they were bruised. Each sample consisted of the soil scraped with a sterile knife from thirty tubers into a new paper envelope. The *p*H value of each field sample was determined and the ranges are given in Table 2. Each soil sample was

used each year to inoculate, through wounds, eighteen healthy tubers; the results are assembled in Table 2. Controls consisted of cut healthy tubers, not inoculated, which remained sound, and of healthy tubers internally inoculated with pure cultures of the fungus, all of which developed dry rot, thus showing that the tubers were susceptible to the disease.

The results show that the dry-rot fungus is widespread in north Cheshire and is viable in field soils having a wide range of *p*H values. They also show a marked contrast between the infectivity of the soil samples scraped from undipped and dipped tubers. The former, in both years, induced severe dry rot attack in the inoculated tubers, except that from farm 7 in 1944. The soil from the dipped tubers, however, induced hardly any disease except on farms 5 and 10 in 1943. It seems reasonable to conclude that dipping killed the fungus almost entirely on the dipped tubers, and this would satisfactorily explain the contrasts between series 3 and 4, and series 6 and 7, in Table 1. The two exceptional cases on farms 5 and 10 are not readily explainable; either the dipping was less effective than on the other farms, which seems unlikely, or recontamination may possibly have occurred after dipping and before the adhering soil was sampled. Whatever the explanation, the result possibly explains the exceptional loss of 21% in series 7 on farm 5 in 1943 (see Table 1).

In soil samples scraped from undipped tubers and kept in sealed envelopes (in the laboratory), the dry-rot fungus has been found to be still viable after 16 months' storage, and even that period may not be a maximum. How long the fungus may remain viable in the soil in the field is not known. The intervals between successive potato crops on the fields where the present investigations were made varied from 1 to 2 years on some, 3 to 6 on others, and at least 9 years on one. On the whole, however, the rotations in the district concerned are relatively short, and it is quite possible that the fungus may survive from one potato crop to the next. Apart from this, however, there is always the possibility that, owing to the prevalence of contaminated seed tubers, both local grown and imported (Small, 1944), the fungus may be freshly introduced into the field soil with the tubers planted.

Dry rot of immature tubers

It is commonly thought that actual decay due to the dry-rot fungus starts in immature tubers less readily and proceeds less rapidly than in those that have been stored for some considerable time. This view is, to some extent, based on the general experience that the curve of dry-rot attack in commercial seed often does not begin to rise until, perhaps, December, and that its peak may not be reached for many weeks afterwards. During this period, internal 'ripening' changes occur within the tubers which may encourage the development of dry rot. On the other hand, during the storage period seed tubers are often 'handled' in one way or another, and this involves a certain amount, sometimes indeed a great deal, of mechanical damage to them. It may be, therefore, that increased attacks may simply run more or less parallel with increased chances of infection, owing to more intimate distribution of contaminating material and increase in the number of suitable ports of entry as time goes on.

Pethybridge & Lafferty (1917) found in laboratory tests with the variety May Queen that internal inoculation with mycelium at intervals from July to February caused little dry rot up to October, more from November to January, and most in February. The writer, using fresh pure cultures (to avoid staling), has confirmed these results with the variety Ninetyfold, although he has also found that in some seasons inoculation of freshly dug tubers in July and August has caused dry rot to develop quickly, whilst in others it has failed to establish infection. Such tests show that the disease appears to be contracted more easily as the tubers become more mature. It may also be added that the rot itself progressed more quickly in mature tubers.

Nevertheless, actual results obtained during the present studies undoubtedly show that dry rot can occur and can be severe in immature tubers. The tubers in series 3, dug in July and deliberately bruised just after lifting, showed losses often exceeding 20% within 7–8 weeks. At farm 3, in 1944, the crop was dug exceptionally early (22 June) and contained but few tubers above seed size, yet, after bruising at lifting time, 34% loss was recorded in mid-October. Control tubers, unbruised either at lifting time or subsequently, remained sound until planting time in the following spring. From these trials, therefore, it seems clear that mechanical damage to the tubers is a more important factor in the incidence of dry rot than degree of tuber maturity. It has been suggested, among others by Samuel (1944), that incipient but invisible infection of the tubers may occur very soon after lifting, but that serious rotting does not develop until internal maturing changes have made headway. There appears, however, to be little or no convincing evidence to support this view. What appears to be more likely is that surface contamination of the tubers exists from the outset, but actual entry of the fungus does not occur to any great extent until mechanical damage to the skin occurs. Even then, possibly because of rapid healing of the wounds, or for some other reason, the fungus may not be able to infect the tubers.

Experiments with more mature seed tubers

The results already discussed were those obtained by examination of the crop of seed tubers up to mid-October. At that time the diseased tubers from the

boxes in series 1–7 in Table 1 were discarded, and the remaining still healthy ones were used in further tests. The lofts in which the tubers continued to be stored were frost-proof and relatively warm, since almost all of them were situated over cowsheds.

(i) *Course of the disease in tubers not further handled.* The healthy tubers in series 3–7 and those in two boxes of each of series 1 and 2 were left undisturbed. Practically no further dry rot appeared in any of these boxes up to planting time in the following spring. Under such conditions, therefore, the loss up to mid-October was the total loss. In series 1 it was practically negligible, showing that little infection occurred in unbruised, undipped tubers, although these were grown in contaminated soil, lifted with a spinner, and stored under conditions favourable for dry rot.

(ii) *Results of further bruising and dipping.* In 1943 and 1944, two boxes of healthy tubers of each of series 1 and 2 were severely and separately bruised in a sterilized sack in October and then returned to their original boxes. In addition, in 1944, two boxes of healthy tubers of each of series 1 and 2 at farms 2, 6, 7 and 8 were dipped in October, severely bruised 7 days later, and then returned to their original boxes. When bruising, adequate precautions were taken in all cases to avoid extraneous contamination. In 1944 the bruised but undipped tubers in series 1 were examined in December, 8–9 weeks after bruising, and heavy losses (41–65%) were found except at two farms. The losses, in both years, in late January, are shown in Table 3. The numbers of tubers examined at each farm in each year varied from 1428 to 5348, the average being a little over 3000. Owing to a misunderstanding the figures for farm 9 are not available. Some further losses occurred at the end of February, but they were slight and do not affect the conclusions to be drawn from the trials.

It will be seen from Exps. 1 and 2 that contaminated, unbruised, undipped tubers, dug in July, stored in boxes and remaining practically sound until mid-October, when severely bruised in that month developed serious dry rot in consequence, after further storage to late January. Reference to Table 1 shows that similar tubers, bruised when dug (series 3) or shortly afterwards (series 6), developed severe dry rot by mid-October. Unbruised controls remained healthy until planting time. It is clear, therefore, that little or no infection occurred in these trials unless and until the tubers had been bruised, and that the time and amount of infection were determined largely by the time and degree of bruising (Small, 1944). In connexion with this conclusion it must be pointed out that in these investigations a considerable variation in the amount of rotted tissue almost always occurred among the tubers of any one series in field trials, as well as in laboratory tests under more controlled conditions. This suggests that either infection or subsequent disease development occurs more readily in some tubers than in others. Nevertheless, the results on the whole support the above conclusion.

TABLE 3. *Effect of bruising and dipping in October on tubers dug in July and stored in boxes; examined late January*

Exp. no.	Year	Treatment as soon as dug in July	October treatment	No. of farm and dry rot (%) 1	2	3	4	5	6	7	8	10
1	1943	Unbruised, undipped (series 1 of Table 1)	Severely bruised, undipped	64	55	75	38	72	63	61	25	52
2	1944	Do.	Do.	29	62	65	78	33	59	24	47	—
3	1944	Do.	Dipped; severely bruised 7 days later	—	41	—	—	—	4	8	0	—
4	1943	Unbruised, dipped (series 2 of Table 1)	Severely bruised, undipped	47	1	4	27	62	41	60	19	40
5	1944	Do.	Do.	9	53	22	31	24	23	1	3	—
6	1944	Do.	Dipped; severely bruised 7 days later	—	10	—	—	—	1	0	3	—

Comparison of Exps. 2 and 3 in Table 3 shows that bruising after dipping in October resulted in very small losses, except on farm 2, no explanation of this aberrant result being at present forthcoming. In two similar trials at Warburton in 1943 the disease was reduced from 25 and 46% in the bruised, undipped tubers, to 2 and 0% respectively in the bruised, dipped ones. Practically no infection occurred in contaminated, unbruised, undipped controls. Delayed dipping (October) of originally unbruised tubers would, therefore, appear to be an effective treatment, even if the tubers become bruised afterwards. Further tests on this point are in progress.

Exps. 4 and 5 of Table 3 show that heavy losses occurred on seven out of nine farms in 1943 and on five out of eight in 1944, as a result of severe bruising in October of previously unbruised, but July-dipped, seed. These losses contrast markedly with the slight

ones shown in Table 1, series 7, where the tubers were severely bruised soon after dipping in July. It is clear that dipping in July did not protect against bruising 3 months later. Several explanations may be suggested to account for this result, but perhaps the most likely one is that after the fungus had been for the most part killed out by dipping in July, recontamination from some source had taken place.

(iii) *Recontamination of tubers.* In this connexion it may be pointed out that Pethybridge & Bowers (1908) showed that the dry-rot fungus may be present in a viable state in the air and the dust on walls and floors of a seed-potato store. The writer collected samples of soil from the floors of six potato lofts in January and February and found, by inoculation into healthy tubers, that the fungus was present and active in all of them. It was still viable in these samples in the following November, hence it seems almost certain that the fungus can survive from one season to the next in such places as lofts, sacks, hampers, etc. The contaminated soil in such lofts is disturbed when men work in or sweep them, and some of it must settle on the stored tubers. Disturbance and contamination also occur, for instance, when bags of freshly arrived contaminated seed are tipped into boxes in the lofts. The writer carried out an experiment in November in which disinfected healthy tubers were cut in halves with a sterile knife and the cut surfaces exposed at once to the results of sweeping the floor of a potato store with a stiff brush. Two hours later, after time for settlement of the dust, seventy of the half tubers were placed in a moist chamber and seventy were left still in the potato store. In due course sixty of the former and thirty-six of the latter developed dry rot. Suitable controls remained sound. Thus, the sweeping of contaminated potato stores immediately after cutting seed tubers may lead to serious loss from dry rot.

Soil scraped from the dipped healthy tubers of Exp. 5, Table 3, just before they were bruised in October 1944, was tested and found to be contaminated on all the farms except 7 and 8. This suggests that the tubers had become recontaminated since they had been dipped in July, because soil scraped from tubers 7–14 days after dipping in July 1944 was, as shown in Table 2, practically free from contamination.

The foregoing evidence clearly indicates that it is risky to keep dipped seed in boxes in potato stores for long periods before they are despatched. Seed tubers that are to be sent away from the farm after undisturbed storage in boxes for some time, which will almost certainly become bruised in transit, might be dipped not when lifted but just before despatch, as that would probably protect them from serious infection. The dipping process might have to be a little prolonged or perhaps a 'wetter' might be added to the fungicidal solution, because such tubers, and the soil that has dried on them, are more difficult to wet thoroughly than still damp ones just after digging.

From Exp. 6, Table 3, it is seen that unbruised tubers dipped in July, dipped again in October and severely bruised 7 days later, developed little dry rot subsequently, though the results on farms 7 and 8 are less striking because at them there was little or no disease in the undipped tubers of Exp. 5 in the same year. In two similar trials made at Warburton in October 1943, dry rot was reduced from 8 to 0% and from 20 to 2%. Thus, in four out of six tests, unbruised tubers, dipped in July at lifting time and stored in boxes in lofts until October and then bruised, developed much dry rot except when redipped before bruising.

Discussion and suggestions

From previous work (Small, 1944) and the present studies it is clear that the primary source of infection of tubers with dry rot is the soil in which the crop is grown. From this source contamination of hampers, boxes, sacks, lofts, etc., recurs frequently, and disinfection of these secondary sources is probably not worth while when dealing with undipped tubers. With dipped seed, however, these secondary sources are by no means negligible, although their relative importance has still to be worked out in detail. For example, when mature Ninetyfold tubers were dipped in Aretan, then dried and severely bruised either in sterilized sacks (control) or in sacks containing a little contaminated soil, the controls, in two tests, developed 8 and 12% dry rot, whereas the tubers from the contaminated sacks developed 58 and 90% dry rot.

Infection rarely occurs, apparently, unless the skins of the tubers become damaged by bruising or some other mechanical means. Thus, the dipping of unbruised tubers, although surface contaminated, would scarcely be worth while if they are harvested into, and stored in, boxes under proper conditions, and can remain in them until planting time. This conclusion, according to the results of the present work, would seem to apply even to crops lifted with a spinner. This would be possible only for home-grown or, at most, locally saved seed. Murphy (1936) records that about 76% of the potato acreage in England and Wales is planted with local seed, and it is significant that serious attacks of dry rot are much less prevalent in such seed than in seed imported from Scotland or Ireland. Nevertheless, losses in such local seed are sometimes heavy. Such losses occurred, for example, when the tubers had been transported from the field to the farm buildings in sacks, or tipped into heaps and graded by machine a week or two later, or machine graded on the field at lifting time. The explanation is obvious; the additional handling of the tubers caused damage and bruising to them, thus enabling the fungus to gain

almost immediate entry. Prompt dipping of such seed would save a lot of it.

Foister & Wilson (1943) have discussed growers' difficulties in dipping seed potatoes, but they nevertheless believe that the practice is likely to extend. Where tubers of susceptible varieties are likely to become exposed to rough handling, the best time for dipping, theoretically, is just before bruising and damage occur, but if this is not possible it should be done immediately afterwards.

Further work is required to determine the most useful and efficient fungicide to employ. Aretan, or other organo-mercury compounds, and formaldehyde (Foister, 1940) have so far given promising results. It seems doubtful whether the protective action of the organo-mercury compound is persistent, for during the present work recontaminated, dipped tubers did contract dry rot if they were subsequently bruised. The poisonous nature of mercury compounds is also somewhat objectionable, and an equally or more efficient, harmless fungicide would be a great advantage.

The work described in the present paper has been connected solely with potatoes stored in boxes; unfortunately, little information is yet available on the incidence of dry rot in tubers stored in clamps in seed-producing areas. Transported seed, reclamped on arrival at destination, need not be considered for the moment. It has been stated (Anon. 1939) that dry rot is negligible in clamps if they are not opened until March, and that dipping the tubers at this time is of no use. Foister (1940) indicates that this disease is absent in constantly closed clamps, but that it may become serious when they have been opened once or several times. No experimental details are given in either of these accounts, and it is, therefore, impossible fully to apprehend the results. If no infection occurs in closed clamps, dipping when the clamp is first opened, and before the tubers are exposed to further rough handling, would appear to be warranted. The writer's trials in 1943 and 1944 (to be published later) showed that dry rot was severe in October, when clamps of Ninetyfold tubers, lifted, bruised and clamped in July, were first opened, whilst it was practically absent at that time in unbruised tubers of the same variety in the same clamp. If it can be established that the dipping of unbruised clamped tubers, delayed until the clamp is opened, is effective in preventing the subsequent development of dry rot, then some of the practical difficulties involved in dipping at lifting time (such as separating seed from ware, lack of sufficient labour at a very busy time, clamping of tubers because sufficient boxes are not available) on large-scale seed-producing farms, where the whole crop (seed and ware) has to be clamped at lifting time, would be overcome. Such delayed dipping might also reduce the risk from recontamination of the dipped tubers if they had to be stored in lofts or clamps before despatch to distant destinations. The dipping might, perhaps, need to be slightly more prolonged and more thorough than with freshly dug, still damp tubers for reasons already given.

My thanks are due to Dr G. H. Pethybridge for assistance in preparing the paper for press. I am indebted to Dr T. W. Walker, Agricultural Advisory Department, Manchester University, for the soil *p*H determinations; also to the following farmers for much help and permission to work on their farms: John Astbury and A. Grice (Halton), Joseph Astbury and W. R. Astbury (Stockham), J. Beckett and J. Walton (Warburton), W. A. Richardson (Grappenhall), J. and T. Peters (Glazebury), and W. H. Winstanley (Lymm).

REFERENCES

Anonymous (1939). Seed testing and plant registration. *Scot. J. Agric.* **22**, 53.

Foister, C. E. (1940). Dry rot diseases of potatoes. *Scot. J. Agric.* **23**, 63.

Foister, C. E. & Wilson, A. R. (1943). Dry rot in seed potatoes. A summary of some recent experiments. *J. Minist. Agric.* **50**, 300.

Murphy, P. A. (1936). Nature and control of potato virus diseases. *Nature, Lond.*, **138**, 955.

Pethybridge, G. H. & Bowers, E. H. (1908). Dry rot of the potato tuber. *Econ. Proc. R. Dublin Soc.* **1**, 547.

Pethybridge, G. H. & Lafferty, H. A. (1917). Further observations on the cause of the common dry rot of the potato tuber in the British Isles. *Sci. Proc. R. Dublin Soc.* **15**, N.S. 21, 193.

Samuel, G. (1944). Some factors affecting the yield of the potato crop. *J. R. Soc. Arts*, **92**, 561.

Small, T. (1944). Dry rot of potato. Investigation on the sources and time of infection. *Ann. appl. Biol.* **31**, 290.

(*Received* 28 *April* 1945)

Field trials of copper fungicides for the control of potato blight

I. Foliage protection and yield

By E. C. Large, B.Sc., A.R.I.C., *Seale-Hayne Agricultural College, Newton Abbot, Devon*

(With 4 Text-figures)

Results are given of eight replicated-plot trials of a number of copper fungicides in comparison with standard Bordeaux mixture under relatively severe conditions of potato blight incidence, in Devon and Cornwall during the years 1941–4.

Recent methods of disease measurement on foliage were applied and developed; characteristic curves were obtained defining the conditions of trial and the relations between spray timing and progress of blight attack. The time interval between half-decay dates for sprayed and unsprayed foliage was taken as a numerical criterion of the direct protective effect of spraying.

A linear relationship was found to hold between probit foliage decay and time, and this was accounted for by the close correspondence of the probit curve to that derived from a growth law, $da = Ca(A-a)dt$, where a denotes the percentage foliage area affected by blight at time t after the beginning of the attack.

Methods were found for preparing dispersible mixtures of bentonite with active copper compounds for use in spraying. With such admixture, to improve adhesion, both cuprous oxide and copper oxychloride preparations nearly equalled Bordeaux mixture at the same copper dosage. Without sticker, and compounded with water-soluble dispersing agents, these materials were equalled by Bordeaux mixture at half their copper dosage.

Suspensions of metallic copper were found to have pronounced fungicidal effect against *Phytophthora infestans*. A bearing of this result on the fungicidal mechanism of cuprous oxide is discussed.

Results from 103 pairs of twice-sprayed and unsprayed plots of main-crop potatoes in Devon and Cornwall in 1941–4 showed a mean prolongation of growth due to spraying of 16½ days, and a mean gain in total yield of 2¾ tons/acre; but the gain in yield resulting from a given prolongation of growth varied greatly, according to the stage of crop development at which the prolongation of growth began.

Spray retention data, for correlation with foliage protection and yields, were obtained in all four years.

Introduction

In the course of a war-time spraying campaign against potato blight in the South-Western Advisory Province, information was needed on the relative practical efficacy of several of the more recently introduced copper fungicides as compared with Bordeaux mixture. Replicated-plot trials were therefore laid down, at several centres, in addition to the many pairs of sprayed and unsprayed strips used for demonstration purposes. The aim was to compare the various spray materials in three respects: (*a*) by their direct protective effect on foliage, (*b*) by their capacity to resist washing off by rain, and (*c*) by the indirect effect of their application on the yield of tubers. Climatic conditions in the region, favouring the early development of potato blight, and the limitation of the sprayings to the two applications which were possible in local farm practice, provided good conditions of trial. As the work went on, from 1941 to 1944, experimental compoundings of spray materials were included in the trials; and a study was made of methods, and units, of disease measurement. By these methods it became possible to define the course of destruction of potato foliage by blight, and the all-important relation between spray timing and protective effect was thrown into prominence.

Sequence of trials

In 1941 a field of 11 acres of Arran Consul, in close proximity to the Dartington meteorological station, near Totnes, Devon, was chosen for an exploratory trial (Table 1 (1)). The field was divided into three blocks, each of nine plots 0·4 acre in area, and eight treatments with one control were randomized in each block. Six sprays and two dusts were included. The sprays were applied with a seven-row horse-drawn sprayer, as nearly as possible at 100 gal./acre, on 30 June and 31 July. The dusts were applied twice only, on the same dates, with a hand-driven dust gun, at the rate of about 25 lb./acre.

The planning of the trial was begun too late to ensure that the seed stock was satisfactory from the point of view of virus disease, and unfortunately by the date of the second spraying most of the plants in blocks 2 and 3 were found to be so severely affected by leaf-roll and mineral deficiencies that the spraying trial on them had to be abandoned. In block 1, however, the plants were reasonably uniform in all plots, with a leaf-roll count of about 5%. On this single block the sprayings were repeated and complete observations were made.

In 1942 careful attention was paid to the virus standing of the seed stocks, and trials were made on

more suitable varieties than Arran Consul, at four centres, chosen as representative of differing climatic conditions, namely, on 'A' certificate Majestic at Dartington, to the south of Dartmoor, with average annual rainfall about 47 in. (Table 1 (4)); on 'A' Majestic at Kentisbeare, on the drier eastern side of Devon (Table 1 (3)); on 'A' King Edward a few miles inland from the Atlantic coast at Bude, where sea fogs are prevalent (Table 1 (5)); and on good once-grown Arran Banner on the cliffs in the same locality (Table 1 (2)). All the trial potatoes in this year were uniform within their respective fields, all the crops showed less than 1% of virus disease (severe mosaic and leaf-roll), and all were free from obvious mineral deficiency symptoms. As it had been found in 1941 that the attempt to spray a large number of replicated plots with a horse-drawn machine was cumbrous, and led to unavoidable inequalities in the rate of application and unnecessary dispersal and increase of soil variation between the plots, all the spraying in 1942 and subsequent years was done by hand with a knapsack machine, and the individual plots were reduced in area to 2 or 3 sq. rods. All trials were laid out to a randomized block design, with three replications of each treatment.

In 1942 the concurrent potato-spraying campaign in the south-west (Beaumont & Large, 1942) had clearly shown that owing to labour shortage and other economic considerations it was impracticable for farmers in the Province to give more than two spray applications. The trials therefore were designed with the definite intention of testing the efficiency of spraying under this practical limitation. Trials with dusts were discontinued, and cuprous oxide and copper oxychloride sprays were chosen for comparison with Bordeaux mixture. To test the effect of spray adhesion, which becomes of extreme importance when only two sprayings can be given, compounded proprietary materials, containing sulphite lye or other water-soluble dispersing agents, were compared with laboratory compoundings of the same active materials with bentonite as a 'sticker'. In this year, also, fine suspensions of metallic copper, with and without added bentonite as adhesive, were first tried for the control of potato blight (Large, 1943).

The unsprayed potatoes at all centres in 1942 were severely attacked by blight, the two sprayings proved to be well-timed in relation to the blight attack, and excellent trials of the effect of two sprayings were obtained.

In 1943, at one centre only, Dartington (Table 1 (6)), trials on 'A' Majestic of materials as in 1942 were continued, with the addition of some lower strengths of Bordeaux mixture, to provide a scale of comparison for other sprays, and of lime sulphur and tetramethylthiuramdisulphide. In this trial, with the blight attack beginning much earlier than in 1942, the second spraying was given too late to be fully effective.

In 1944 the trials were continued on 'A' Majestic at Dartington (Table 1 (7)) and Dunsford (Table 1 (8)), with four replications of each treatment. Two new treatments were included, i.e. potash Burgundy (because of its possible value for the simultaneous control of blight and of leaf-scorch due to potash deficiency); and 'complete protection', an additional type of control in spraying experiments advocated by Murphy (1939), achieved by frequently repeated applications of ½% Bordeaux mixture. In 1944 the blight attack proved very similar to that in 1942, and the second spraying was given too early.

Materials

(1) *Traditional and proprietary mixtures.* The standard spray fluid used throughout was 1% Bordeaux mixture (4.5.40) freshly prepared from granulated copper sulphate crystals and hydrated lime. Half and ¼% mixtures (2.2½.40) and (1.1¼.40) were included as further standards of comparison, and ½ and ¼% mixtures with excess lime (2.5.40) and (1.5.40) were used to test the possibility of reducing the amount of copper required for effective control provided that the normal amount of lime was retained to act, perhaps, as a mortar-like adhesive agent.

A proprietary Burgundy mixture (Jeunite), containing 15% Cu, was used at 7 lb./40 gal. A cuprammonium compound (Fungex) containing 8·75% Cu w/v was used at 5 pt./100 gal. A copper-lime dust (16% Cu) was applied at 25 lb./acre.

Copper oxychloride, in assumed combination with sulphite lye or other water-soluble dispersing agent, was represented by Coppesan and Soltosan each containing 50% Cu and used at 5 lb./100 gal., and also by the liquid preparation Bouisol, sp.gr. 1·4, containing 15% Cu by wt., and used at 5 pt./100 gal. A mixed Bouisol and white-oil emulsion, containing 3·04% Cu w/v, was used at 10 pt./100 gal.

Cuprous oxide in assumed combination with a water-soluble dispersing agent was represented by Perenox, containing 50% Cu and used at 5 lb./100 gal. A cuprous-oxide dust, Perelan, containing 17·5% Cu was used at 25 lb./acre.

Sulphur sprays included in one of the trials were lime sulphur sp.gr. 1·30, used at 1 in 50, and a preparation of tetramethylthiuramdisulphide (R 1104 × 32) at 1 in 400.

Potash-Burgundy mixture was made up from 10 lb. copper sulphate crystals and 7½ lb. potassium carbonate (95% K_2CO_3) per 100 gal.

(2) *Dispersible bentonite mixtures.* Materials containing cuprous oxide and copper oxychloride in combination with bentonite for improved adhesion were prepared in the laboratory. Dry mixtures of the active substances with bentonite formed sticky pellets on the addition of water and could not be suspended for use in spraying without prolonged

soaking or kneading. In the copper-oxychloride material 'M', prepared according to British Patent 493,148 (Large, 1938), this difficulty was overcome by incorporating a considerable proportion of inert anhydrous sodium sulphate. To prepare material for 100 gal. of spray fluid, 10 lb. copper sulphate crystals, 8½ lb. crushed washing soda, 1¼ lb. common salt and 1¼ lb. bentonite were mixed together and warmed. When the vigorous reaction was complete, the cake was dried to about 22% Cu and crumbled. This product dispersed readily in water as required for use. To reduce the proportion of inert anti-balling agent required, and to produce a dispersible bentonite mixture that could be added to any finely divided active copper material, the effect of adding electrolytes of higher valency, e.g. alum, citric acid, and pyrophosphates was tried. It was found that if 96 parts by weight of bentonite were blended, dry, with 2 parts of aluminium sulphate and 2 parts of finely ground washing soda, the difficulty of balling was overcome without adversely affecting the stability of the final suspension. The material 'D', yellow cuprous oxide with bentonite, was prepared by mixing 3 lb. yellow cuprous oxide (Lennig) 85·4% Cu, with 5 lb. of the 96–2–2 bentonite mixture, per 100 gal. In use, the dry mixture was first creamed with a *little* water, and the cream was then poured into the rest of the water in the sprayer. Employed in this way the effect of the added electrolytes was to inhibit the swelling of the bentonite until the individual particles were well dispersed.

(3) *Designations of sprays and copper dosages used.* The following abbreviations are used, throughout this and the succeeding paper (Large *et al.* 1945), for the various materials employed:

B	1% Bordeaux mixture (4.5.40)	0·25% Cu
½B	½% Bordeaux mixture (2.2½.40)	0·125
¼B	¼% Bordeaux mixture (1.1¼.40)	0·063
½BX	½% Bordeaux mixture with excess lime (2.5.40)	0·125
¼BX	¼% Bordeaux mixture with excess lime (1.5.40)	0·063
KB	1% Potash-Burgundy mixture (4.3.40)	0·25
J	1% Burgundy mixture (Jeunite) (7½.40)	0·25
D	Yellow cuprous oxide with bentonite (3.5.100)	0·25
½D	Yellow cuprous oxide with bentonite (3.5.200)	0·125
P	Yellow cuprous oxide (Perenox) (2.40)	0·25
K	Metallic copper with bentonite (2½.5.100)	0·25
H	Metallic copper with sulphite lye (2½.1.100)	0·25
M	Copper oxychloride with bentonite (vide supra)	0·25
C	Copper oxychloride (Coppesan) (2.40)	0·25
S	Copper oxychloride (Soltosan) (2.40)	0·25
Bsl	Copper oxychloride (Bouisol) (vide supra)	0·125
Bwo	Copper oxychloride (Bouisol and white oil)	0·038
F	Cuprammonium compound (Fungex) (vide supra)	0·055
CLD	Copper-lime dust 16% Cu at 25 lb./acre	(0·40)
PN	Cuprous oxide dust (Perelan) 17·5% Cu at 25 lb./acre	(0·43)
L	Lime sulphur 1 in 50	
T	Tetramethylthiuramdisulphide (vide supra)	

All the spray fluids were applied at approximately 120 gal./acre for the first and 160 gal. for the second spraying except where otherwise stated.

(4) *Suspensions of metallic copper.* The preparation of suspensions of metallic copper suitable for use in spraying was attempted by several methods, e.g. treatment of cuprous oxide with dilute sulphuric acid, reduction of cupric salts by sodium hydrosulphite, and displacement with iron or zinc. A preparation made by the last-named method was used in trial 5 (Table 1), but for the rest of the trials, technical (B.D.H.) hydrogen-reduced copper metal was employed. The material as received was lightly triturated in a mortar to break down aggregates of particles, and then bolted through silk. To produce material for 100 gal. of spray fluid, 2½ lb. of the fine metallic powder was blended dry with 5 lb. of 96–2–2 bentonite mixture for material K, and with 1 lb. of dried and ground sulphite lye for material H. The copper particles in these mixtures were relatively coarse, mostly within the size range 2–5 μ. The question of oxidation of the copper particles is discussed infra.

METHODS

Measurements of the extent of blight injury to the potato foliage at successive dates were made (*a*) to define the conditions of trial, in relation to blight attack, and (*b*) to assess the direct protective effect of the treatments on the foliage. Spray-retention determinations were made at Dartington in each of the four years; and yield data, from replicated plots, were obtained at all centres.

Measurement of disease intensity on the foliage. Marsh *et al.*, in 1937, employed a scale of stages of blight infestation designated by numerals, 0, 2, 4, ..., 10, each stage being defined by a brief verbal description of the condition of the foliage (e.g. 2 = blight lesions scarce), without attempt, at that time, to relate the grades directly to the percentage of the foliage area rendered useless for further

photosynthesis. In 1941 co-operative work was initiated by the Disease Measurement Sub-Committee of the British Mycological Society to establish a more precise scale of grading (Moore, 1943). Six grades were then proposed, as follows:

0 = no blight,
1 = blight lesion on one leaflet per plant,
2 = lesions on 2–10 leaflets,
3 = lesions on 10–50 leaflets,
4 = one-third of foliage area destroyed,
5 = two-thirds of foliage area destroyed,
6 = complete defoliation.

This scale was used as the basis of assessment in the exploratory trial in 1941. In obtaining the mean assessment for an entire plot, a number of random samples of ten consecutive plants within the plot were examined. It was soon found possible to discard the use of an arbitrary scale of grades, and to express mean assessments for whole plots in terms of percentage foliage area destroyed. In 1942 the B.M.S. Committee's recommendations were on this basis, with categories of 0·0, 0·1, 1·0, 25, 50, 75 and 100% foliage destroyed.

In all the present trials, after 1941, the method adopted was to examine a number of typical plants in each plot, at each date of inspection, and to estimate the total area of the foliage in terms of an equivalent number of average-sized primary leaflets. The decayed area was then estimated in the same terms, i.e. by the number of average-sized primary leaflets that the blight lesions present would completely cover. Thus a well-grown Majestic plant, in full leaf-deckage in August, might possess the equivalent of 500 primary leaflets. An average of one blight lesion (occupying about half the area of a single average leaflet) per plant at this stage would therefore be taken as equivalent to 0·1% of the leaf area affected. To assist the eye in these estimations, typical plants were stripped of their leaves at several stages of growth, the leaves were sorted into categories, photographed, and measured with a planimeter. With such aids it was found that very close estimates of percentage foliage destruction could be made in the earlier stages of the attack (up to about 100 discrete blight lesions per plant). In later stages, when some whole leaves had totally withered or fallen, a wider margin of error was inevitable; but it was found that the following stages could be recorded, with confidence that the true figure lay between the figures immediately above and below that which was taken:

0·1, 0·5, 1·0, 2·5, 5·0, 10, 15,
25, 33, 50, 66, 75, 85, 95, 100.

Independent assessments for each replicated plot were made on each date of inspection, and the mean assessments are given in Table 1.

Estimation of yield. The tubers from two sample strips of ten consecutive plants in each replicated plot were lifted by hand, graded and weighed. The position of the sample strips within the plots was located at random by throwing a cane. The length of drill occupied by each ten plants was measured, and the mean length for all the sample strips in the field, usually between fifty and sixty, was taken in the conversion of yields to tons/acre. The mean spacing between the drills, also required for the conversion, was found by measurements across twelve drills in a number of places in the field. The proportion of blighted tubers in all the trials was low, usually less than ½%, and mean *total* yields are given in Table 1, as it is considered that the total yield provides the best measure of the indirect effect of spraying in terms of yield.

Significant differences between the mean total yields were obtained by the analysis of variance, and are entered in Table 1.

Results

The results obtained in all the trials for the four years 1941–4 are assembled in Table 1, together with brief references to the location of the trial sites, varieties and seed stocks, and dates of spraying.

The derivation of the figures for half-decay dates, and days of growth gained by spraying, is dealt with below. The whole of the rest of this paper constitutes a discussion of the results, which is concluded by a summary of the critical comparative data for the six principal types of spray material tested (Table 2).

Spray timing

In Fig. 1 curves for mean percentage foliage destruction by blight in plots left unsprayed, and in plots twice sprayed with standard Bordeaux mixture, are plotted to a time base, for the trials at Dartington in four consecutive years. The rainfall, minimum screen temperature, and relative humidity at 3 p.m. (G.M.T.) for each day, over all the trial periods, are also shown, and the dates on which the sprayings were given are indicated by symbols at the tops of the diagrams.

It will be seen that in all the trials the progress of blight on the sprayed foliage followed much the same course as on the unsprayed, but after a time interval the length of which depended essentially on the timing of the spray applications in relation to that of the blight attack.

In 1941 a month of heavy rain (August) intervened between the second spraying and the visible beginning of the blight attack. Both sprayings in this year were given about a month too early. In 1942 the spray timing was almost ideally related to that of the blight attack and the greatest prolongation of useful growth was obtained. In 1943 the second spraying was definitely too late, since it was given

TABLE 1. *Summary of results obtained in all trials, 1941–4*

Treatment and dates of spraying	Percentage of foliage area destroyed or covered by blight lesions							Date of half-decay of foliage ($\frac{1}{2}D$)	Days of growth gained ($\frac{1}{2}D_s - \frac{1}{2}D_0$)	Total yield tons/acre
(1) Arran Consul, Dartington, 1941										
30. vi and 31. vii	28. viii	10. ix	25. ix	1. x						
B	—	0·1	5	5				6. x	14	16·0
P	0·1	1·0	33	50				1. x	8	15·1
J	—	0·1	5	33				6. x	14	15·0
C	0·1	0·1	25	50				1. x	8	14·5
Bsl	0·1	0·1	50	50				25. ix	3	14·5
S	0·1	0·1	25	50				1. x	8	14·5
CLD	0·1	0·1	66	66				22. ix	0	13·2
PN	0·1	1·0	50	66				25. ix	3	13·2
Unsprayed	1·0	10	66	75				22. ix	0	13·2
(2) Arran Banner, once-grown, on cliffs at Bude, 1942										
22. vi and 20. vii	6. viii	25. viii	9. ix	8. x						
D	—	0·1	25	100				(14. ix	15	15·4
B	—	0·1	27	100				13. ix	14	14·4
P	—	0·1	33	100				12. ix	13	14·2
K	—	0·2	41	100				11. ix	12	13·8
Unsprayed	0	7	100	100				30. viii	0)	10·6
								Significant difference ($P = 0·05$)		1·4
(3) Majestic, 'A' Irish, Kentisbeare, East Devon, 1942										
1. vii and 29. vii	19. viii	29. viii	5. ix	12. ix	22. ix	29. ix	7. x			
B	—	0·1	5	28	44	64	87	23. ix	23	16·1
M	—	0·1	4	25	55	66	90	21. ix	21	16·1
D	—	0·1	4	25	55	69	98	21. ix	21	14·7
C	—	0·1	7	50	72	81	98	16. ix	16	15·0
P	—	0·2	7	50	69	75	98	16. ix	16	14·2
K	—	0·2	15	50	82	87	98	13. ix	13	14·3
H	—	0·1	8	58	87	95	100	11. ix	11	14·0
Unsprayed	0·4	38	93	97	100	100	100	31. viii	0	11·6
								Significant difference ($P = 0·05$)		1·8
(4) Majestic, 'A' Scotch, Dartington, 1942										
10. vii and 13. viii	11. viii	18. viii	24. viii	28. viii	3. ix	14. ix	21. ix			
D	—	—	—	0·2	5	33	89	15. ix	21	14·3
B	—	—	0·1	0·1	7	47	89	14. ix	20	14·3
C	—	—	0·1	0·4	7	50	97	14. ix	20	14·3
P	—	—	0·4	0·4	8	55	97	13. ix	19	14·3
J	—	0·1	0·2	2	12	61	97	11. ix	17	14·0
S	—	0·1	0·1	0·9	12	65	97	11. ix	17	14·3
½B	—	0·1	0·1	0·7	12	72	97	11. ix	17	14·6
K	—	0·1	0·2	0·7	12	75	100	11. ix	17	14·6
H	—	0·1	0·2	0·1	20	75	100	11. ix	17	14·6
Unsprayed	0·1	5	43	75	98	100	100	25. viii	0	11·1
								Significant difference ($P = 0·05$)		1·6
(5) King Edward, 'A' Devon, inland near Bude, 1942										
22. vi and 20. vii		6. viii	25. viii	9. ix						
B		—	5	100				(3. ix	15	13·5
K		—	5	100				3. ix	15	13·9
Unsprayed		0·1	100	100				19. viii	0)	11·4
								Significant difference ($P = 0·05$)		0·9

TABLE 1 (*continued*)

Treatment and dates of spraying	Percentage of foliage area destroyed or covered by blight lesions				Date of half-decay of foliage ($\frac{1}{2}D$)	Days of growth gained ($\frac{1}{2}D_s - \frac{1}{2}D_0$)	Total yield tons/acre
(6) Majestic, 'A' Devon, Dartington, 1943							
5. vii and 6. viii	7. viii	19. viii	26. viii	13. ix			
D	1	22	27	66	5. ix	16	14·6
¼B	4	33	27	72	3. ix	14	14·2
½B	2	26	36	71	3. ix	14	13·8
K	3	37	44	74	30. viii	10	13·8
C	3	34	47	66	31. viii	11	13·7
Bwo	1	28	36	84	31. viii	11	13·6
B	2	24	36	66	3. ix	14	13·4
P	3	23	35	71	3. ix	14	13·1
½D	2	23	34	72	3. ix	14	12·5
T	13	27	64	98	23. viii	3	12·3
F	2	23	36	82	31. viii	11	12·2
L	6	30	47	98	25. viii	5	12·1
Unsprayed	18	47	65	100	20. viii	0	11·4
					Significant difference ($P=0.05$)		1·8
(7) Majestic, 'A' Devon, Dartington, 1944							
29. vi and 25. vii	4. viii	11. viii	23. viii	4. ix	11. ix	27. ix			
½B × 6	—	—	0·7	10	18	66	22. ix	30	15·8
B	—	—	0·5	50	75	100	4. ix	12	13·8
D	—	—	0·5	50	73	100	4. ix	12	13·5
½B	—	—	0·5	66	88	100	2. ix	10	13·3
½BX	—	—	1·2	66	82	100	2. ix	10	13·9
P	—	—	1·5	66	91	100	2. ix	10	13·0
KB	—	—	0·5	50	81	100	4. ix	12	13·7
Unsprayed	0·1	0·5	60	95	100	100	23. viii	0	11·2
							Significant difference ($P=0.05$)		1·4
(8) Majestic, 'A' Devon, Dunsford, 1944							
30. vi and 27. vii	10. viii	25. viii	5. ix	25. ix	12. x	30. x			
½B × 6	—	—	—	12·5	50	100	12. x	40	17·4
B	—	—	0·1	65	100	—	23. ix	21	16·1
D	—	—	0·1	77	100	—	21. ix	19	15·9
½B	—	—	0·3	81	100	—	20. ix	18	16·2
½BX	—	—	0·1	77	100	—	21. ix	19	14·5
P	—	—	0·3	76	100	—	21. ix	19	14·9
KB	—	—	0·1	75	100	—	21. ix	19	15·1
¼B	—	—	0·7	84	100	—	19. ix	17	15·4
Unsprayed	0·1	5	69	100	—	—	2. ix	0	13·5
							Significant difference ($P=0.05$)		1·5

after the disease had become established on the sprayed plots, and over 1% of the foliage was already affected. In 1944 a cold dry spell of 3 days in the third week of July almost certainly checked the build-up of inoculum preceding the visible attack, and the second spraying was too early.

The trials in the 4 years were therefore well representative of conditions of early, late, and approximately correct spray-timing inevitably occurring in practice when only two sprayings can be given.

Measurement of prolongation of growth due to spraying

In the older potato-spraying trials it was customary to measure the approximate prolongation of growth due to spraying by the time interval between the end-points of the attack on sprayed and unsprayed plots, or by such general statements as 'the sprayed were still green *n* weeks after the unsprayed were dead'. With the introduction of methods of disease measurement on the foliage, Marsh *et al.*

(1937) and others have taken the mean of foliage decay estimates on successive dates as an inverse measure of the degree of protection obtained with various treatments. Such means, of estimates not even equally spaced in time, are weighted by the proportion of early and late observations that happen to be included, and they constitute mere arbitrary 'marks', comparable only within a particular trial.

In the present work it has been considered preferable to obtain the date of 'half-decay' of the foliage ($\frac{1}{2}D$) by plotting the foliage decay curves, and then taking the time interval ($\frac{1}{2}D_t - \frac{1}{2}D_0$) between the dates of half-decay for foliage given spray treatment t, and unsprayed foliage, as the fairest single numerical criterion of the direct effect of spray treatment t (Fig. 2 *a*).

Use of probit transformation. In plotting the foliage-decay curves it was found convenient to employ the probit transformation of Bliss (1935). Fig. 2 *b* shows the same curves as in Fig. 2 *a* after transformation of the ordinates to probit units. The probit table was used, in effect, in place of a set of universal french curves, for reducing sigmoid shapes to straight lines, or lines of shallow curvature, without assumption (for which no justification could at first be seen) that the normal course of foliage destruction by blight would by any means follow the probability-integral law on which the probit transformation is based. But as, for example, in Fig. 2, which gives the characteristic curves for Dartington in 1942, it was found that there was often an approximately linear relationship between probit-leaf

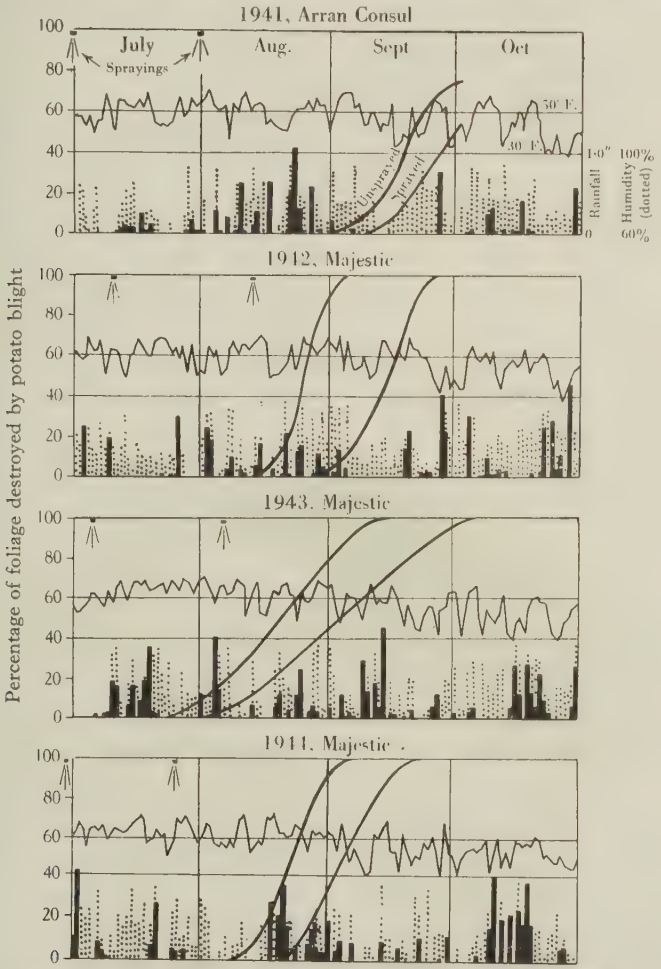


Fig. 1. Progress of foliage destruction by potato blight, in relation to dates of spraying, daily minimum screen temperatures, rainfall, and 3 p.m. percentage humidity, at Dartington, south Devon, 1941–4.

destruction and time.* In *all* the present trials this relationship held for *unsprayed* foliage, within the limits of accuracy of the original assessments; and where departures from linearity occurred among the curves for *sprayed* foliage, some marked lack of uniformity in weather conditions over the period of attack could be associated with the deviation.

Biological significance of linear relationship between probit foliage decay and time

Jensen & Rostrup (1892), when working on the control of blight by the method of reducing the number of primary foci through heat treatment of seed potatoes, accounted for the slow development of the blight attack in its previsible stages by speculations involving units of infected area and the compound interest law. If at any early stage of the attack there was one small unit of the total foliage area on which the fungus was sporing, then, after a unit interval of time—the length of which depended on weather conditions—each unit of infected area would give rise to r units of infected area, and after t intervals of time the infected area would be given by

$$a = Ke^{rt}. \qquad (1)$$

This simple relationship may hold good in the early stages of attack, while the total foliage area A remains almost infinitely great in relation to the infected area a. But as the attack progresses less and less of the foliage remains susceptible to infection, and the rate of increase of the diseased area will be proportional both to the area already infected and to the area remaining susceptible to infection:

$$\frac{da}{dt} = Ca(A-a). \qquad (2)$$

This growth law is the same as that obtained by M'Kendrick & Kesava Pai (1911) in the somewhat comparable problem of the increase of bacteria in a limited medium. Integrating with respect to t, writing A as unity, and assuming for convenience of calculation that the infected area at the beginning of the attack is 0·0001 (the equivalent of one completely diseased leaflet in 10,000), then the relation between a and t becomes

$$a = \frac{0{\cdot}0001}{e^{-rt} - 0{\cdot}0001}. \qquad (3)$$

Justification for the use of the probit transformation for potato blight progress curves may now be obtained by demonstrating that the curve given by the growth law (3) can be closely fitted by that of the probability integral:

$$a = \frac{1}{\sqrt{2\pi}} \int e^{-\frac{1}{2}t^2}. \qquad (4)$$

If r in (3) be given the value 1·842, then the half-decay point ($a = 0{\cdot}5$) is reached when $t = 5$ as in the probit scale, and corresponding ordinates of the two curves are as follows:

Values of (t)	Values of (a): From growth function (3)	Values of (a): From probability function (4)
2·0	0·40	0·14
2·5	0·98	0·62
3·0	2·44	2·30
3·5	5·95	6·70
4·0	13·67	15·90
4·5	28·40	30·80
5·0	50·00	50·00
5·5	71·60	69·20
6·0	86·33	84·10
6·5	94·05	93·30
7·0	97·56	97·70
7·5	99·02	99·38
8·0	99·60	99·86

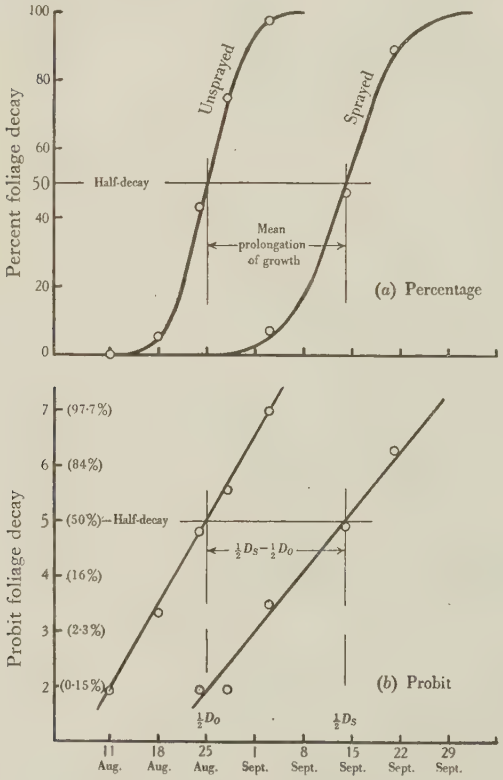


Fig. 2. Characteristic decay curves for potato foliage during a potato blight epidemic, showing half-decay dates, mean prolongation of growth due to spraying, and reduction of the decay curves to straight lines by use of the probit transformation (Dartington, 1942).

* If, as is here indicated, the probit curve closely approximates to that of the normal course of foliage destruction during a blight attack, then the natural stages of leaf destruction, equally spaced in time, would be:

2	3	4	5	6	7	8
0·1	2·3	16	50	84	97·7	99·9 %

and the whole course of a blight epidemic could be completely described by giving the half-decay date, and the slope of the probit foliage decay line.

A closer correspondence could be obtained by using statistical methods of curve fitting to determine the constants a_0 and r in (3), but the above treatment is sufficient to show that the probability curve crosses the growth curve at three points, and that except at their extremities the two curves can correspond so closely that both will give an equally good fit to actual blight progress curves within the limits of accuracy of the original observations.

The function of spraying

The absolute value of the t unit per unit probit increase of foliage area affected by blight, i.e. from about 2 to about 16%, or from 16 to 50%, or from 50 to 84%, was about 4½ days for the unsprayed plots at Dartington in 1942, with favourable conditions for both spore formation and incubation, recurring over the whole period of the attack. Crosier (1934) has shown that with temperatures of 20–25° C. the period of incubation is 3–4 days, and active sporulation can begin on the new lesions within 12–24 hr. Hence each stage in the probit (or growth unit) scale of foliage destruction would appear to correspond approximately to one generation of the blight fungus from spore to spore on the potato leaves.

The interval between the beginning of visible attack (taken as 0·1%) on the Bordeaux-sprayed and unsprayed plots at Dartington in this trial was about 13 days, equivalent to approximately three generations of spores. Thus, it may be said that the effect of spraying in this trial was to retard the attack by three generation periods. The close conformity of the observed progress of blight on the foliage with the growth law, $da = Ca(A-a)dt$, suggests that a delay of three generation periods in the early stages of the attack will result in at least the same delay in all the later stages, and this would appear to be the principle underlying the protection of the foliage by spraying.

The fact that the characteristic curves for sprayed and unsprayed foliage in all the present trials were so nearly parallel, further suggests that spray deposits exert their principal protective effect in the previsible or very early stages of the attack, and that after about 1% of the foliage is visibly affected the presence of spray deposits on the leaves may have little retarding effect on the course of their destruction.

Influence of prolongation of growth on yield of tubers

Fig. 3 shows the progress of crop development in three lifting trials in which the foliage was kept completely free from blight by frequent applications of a copper fungicide at weak dosage. One of the curves (Murphy, 1939) is for Up-to-Date, grown at Glasnevin in 1937, the others are for Majestic, grown at Pinhoe and at Dunsford, in Devon, during 1943 and 1944 respectively.

Consideration of these curves alone is sufficient to show that the value of a given prolongation in growth of haulm due to spraying will vary greatly according to the stage of tuber development from which the prolongation starts, and according to the level of potential yield of the crop as determined by soil, manurial and climatic factors. It will also vary according to the alternation of wet and dry spells over the growing period.

No consistent relation between prolongation of foliage growth and increase of yield from spraying is, therefore, to be expected. How widely the indirect effect of foliage protection on yield may vary in practice was most graphically revealed by the results of all the 103 demonstration and replicated-plot trials carried out in Devon and Cornwall during

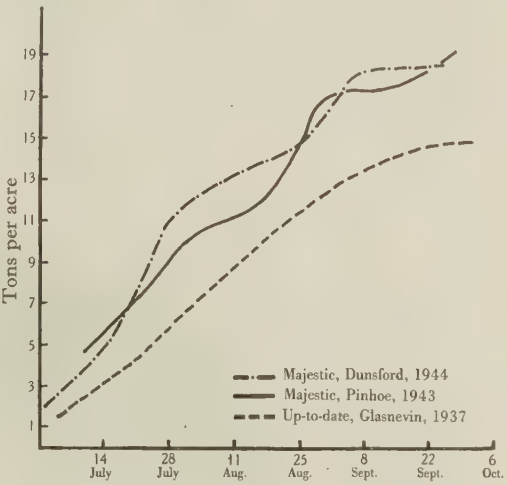


Fig. 3. Progress of tuber development in crops kept completely free from potato blight.

the years 1941–4. The mean prolongation of foliage growth due to spraying ($\frac{1}{2}D_s - \frac{1}{2}D_0$), and the yield, for all these trials were obtained by the methods described in this paper, and the results are shown in the scatter diagram (Fig. 4). The mean prolongation of foliage growth (due to two sprayings) over all the trials was 16½ days, and the mean gain in yield was 2¾ tons/acre. But, in individual trials, a gain of about 16½ days in growth of haulm was associated with anything from 1 to over 4 tons/acre increase in yield, and the scatter of the points was much too great to justify the calculation of a regression coefficient.

Comparative efficacy of spraying materials

Potato yields are affected by so many factors, other than protection of the foliage, that yield figures are of little critical value in comparative trials of spraying materials. This realization has been stressed

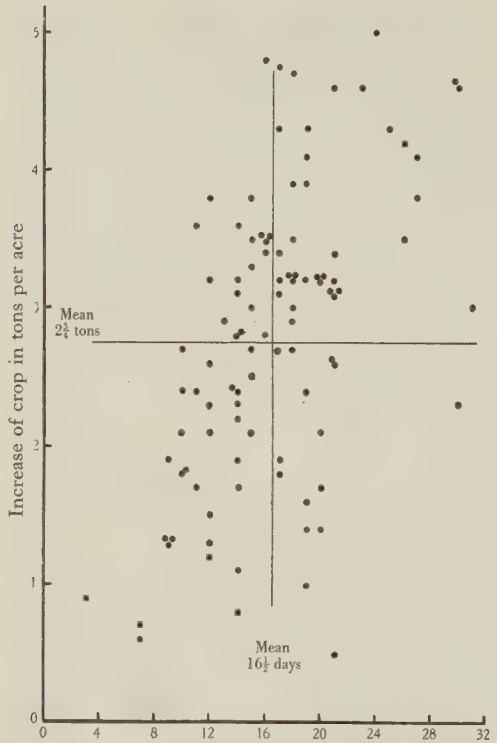


Fig. 4. Prolongation of growth of haulm due to spraying, and corresponding gain in yield of tubers, in 103 spraying trials on main-crop potatoes in Devon and Cornwall, 1941–4.

repeatedly by Marsh, Martin, and their co-workers at Long Ashton, among others, during the past 10 years, and all the results of the present work tend to substantiate and confirm it. As may be seen from Table 1, there was, in all the trials, a highly significant (and economically important) difference between the yields from sprayed and unsprayed plots, but the differences in yield between treatments were often negligible and at best barely significant.

There were, however, obvious differences in the direct protective effects of the several sprays on the potato foliage, and these differences were fairly consistently related to the spray retention factor. In Table 2 the mean data for foliage protection, with the six principal types of spray material tested, are assembled with the corresponding mean figures for spray retention, as obtained in Table 4 et seq. of the succeeding paper (Large *et al.* 1946).

The lasting protective effect of Bordeaux mixture (4.5.40) was once again confirmed; but cuprous oxide with the addition of a bentonite sticker nearly equalled Bordeaux mixture at the same copper dosage. Cuprous oxide and copper oxychloride, compounded with water-soluble dispersing agents and without stickers, were equalled by Bordeaux mixtures at half their copper dosage.

Metallic copper, compounded with bentonite, but in relatively coarsely divided form, was not greatly inferior to very finely divided cuprous oxide without sticker at the same copper dosage. The metallic copper used was kindly examined by Martin and Wain and found to contain, in fact, 44·22% Cu, 45·93% Cu_2O, and 9·85% CuO (in litt. 1943). It was expected that any form of powdered copper metal would be oxidized to a considerable extent, if not before application at best shortly after exposure on the leaves. The purpose of including this material in the trials, however, was to show that the products resulting from atmospheric oxidation of copper metal are fungicidally effective, and to challenge the view that in the mechanism of the fungicidal action of cuprous oxide, only one half of the copper becomes available as the cupric ion, while the other half is deposited as more or less inert copper metal. This may perhaps occur as a transitory phase in a chain of reactions; but any metallic copper so formed must be expected to oxidize very rapidly on exposure, with the result that in practice the whole of the copper can be effective.

TABLE 2. *Foliage protection in relation to spray retention*

Variety: Majestic

	Copper dosage %	Mean prolongation of growth due to two sprayings in days					
		1942			1943	1944	Mean percentage spray retention
Trial no. ...		(3)	(4)	(6)	(7)	(8)	
1% Bordeaux mixture	0·25	23	20	14	12	21	38
Cuprous oxide with bentonite (D)	0·25	21	21	16	12	19	32
½% Bordeaux mixture	0·125	—	17	—	10	18	25
Do. with excess lime	0·125	—	—	14	10	19	
Cuprous oxide without sticker (P)	0·25	16	19	14	10	19	18
Copper oxychloride without sticker (C)	0·25	16	20	11	—	—	12
Metallic copper with bentonite (K)	0·25	13	17	10	—	—	17

The author gratefully acknowledges the help of Mr A. Beaumont of Seale-Hayne Agricultural College, throughout the progress of this work; of Dr G. H. Pethybridge, of Bodmin, both in the field and during the preparation of the paper for publication; of Dr H. Martin and Dr R. L. Wain, of Long Ashton Research Station, in providing some of the cuprous oxide used, and in analysing preparations of metallic copper; and, finally, of Mr R. Hawken, of Seale-Hayne College, in the careful spraying and lifting of several hundreds of trial plots.

REFERENCES

BEAUMONT, A. & LARGE, E. C. (1942). Potato spraying in the south-west, 1941. *J. Minist. Agric.* **48**, 235.

—— (1944). Potato spraying in the south-west, 1942 and 1943. *J. Minist. Agric.* **51**, 71.

BLISS, C. I. (1935). The comparison of dosage-mortality data. *Ann. appl. Biol.* **22**, 307.

CROSIER, W. (1934). Studies in the biology of *Phytophthora infestans* (Mont.) De Bary. *Mem. Cornell. Univ. agric. Exp. Sta.* no. 155.

JENSEN, J. L. & ROSTRUP, E. (1892). Report on recent experiments in checking potato disease in the United Kingdom and abroad (C-6647). *Bd. Agric. G.B. & I.*

LARGE, E. C. (1938). Improvements relating to fungicidal compositions. British Patent, No. 493,148.

—— (1943). Control of potato blight (*Phytophthora infestans*) by spraying with suspensions of metallic copper. *Nature, Lond.*, **151**, 80.

LARGE, E. C., BEER, W. J. & PATTERSON, J. B. E. (1946). Field trials of copper fungicides for the control of potato blight. II. Spray retention. *Ann. appl. Biol.* (in the Press).

MARSH, R. W., MARTIN, H. & MUNSON, R. G. (1937). Studies upon the copper fungicides. III. *Ann. appl. Biol.* **24**, 853.

M'KENDRICK, A. G. & KESAVA PAI, M. (1911). The rate of multiplication of micro-organisms: a mathematical study. *Proc. R. Soc. Edinb.* **31**, 649.

MOORE, W. C. (1943). The measurement of plant diseases in the field. *Trans. Brit. mycol. Soc.* **26**, 28.

MURPHY, P. A. (1939). A study of the seasonal development of the potato in relation to blight attack and spraying. *Sci. Proc. R. Dublin Soc.* **22** (N.S.), 69.

(*Received* 4 *June* 1945)

Blind-seed disease of rye-grass (*Phialea temulenta* Prill. & Delacr.)

BY E. L. CALVERT AND A. E. MUSKETT

Plant Disease Division, Ministry of Agriculture, The Queen's University of Belfast

(With Plates 10 and 11 and 1 Text-figure)

A short account of the present state of knowledge regarding the disease is given. Methods for the examination of seed samples for the estimation of infection with *Phialea temulenta* are described; the most suitable is the examination of each seed microscopically after the removal of one or both glumes, for the presence of macroconidia. The causal organism was isolated by germinating macroconidia and ascospores and also from mycelium within the seed. Its growth on media confirmed previous indications that *Pullularia pullulans* was not the parasite responsible for the disease. Growth in culture is slow, and the production of numerous mucilage-covered macroconidia, the formation of which was observed, gives a slimy appearance to the culture on most media.

The fungus and its life history are described. Inoculation experiments carried out in 1940 and 1941 proved the pathogenicity of *Phialea temulenta* and the saprophytic nature of *Pullularia pullulans*. Italian rye-grass, commercial perennial rye-grass and two strains of pedigree perennial rye-grass were all found to be susceptible to attack when inoculated at flowering time with either macroconidia or ascospores. Using commercial perennial rye-grass the amount of infection was very much reduced when inoculations were carried out after flowering had ceased. The correlation coefficient between percentage infection and germination was calculated from the results of inoculation experiments carried out in 1941 and for samples of pedigree perennial rye-grass (S 24) seed received for examination in 1940, 1942 and 1943. In all cases there was a highly significant, negative correlation indicating that infection with *Phialea temulenta* resulted in reduced germination. The fungus has been isolated from *Festuca ovina* L., *Agrostis canina* L., *A. palustris* Huds., *Poa pratensis* L., *Holcus lanatus* L. and *Cynosurus cristatus* L., while *Festuca elatior* L. and *Poa trivialis* L. were successfully inoculated in 1943 using macrospores of the fungus.

The control of the disease by the breeding of resistant strains and by crop management is discussed. There is little possibility of *Phialea temulenta* being viable in the seed if an over-year sample is sown. In field experiments hot-water treatment of the seed prevented the production of apothecia, but infection occurred owing to the dispersal of ascospores from other plots. A later experiment indicated that the disease is widespread in pastures, waste ground and hedgerows so that the sowing of disease-free seed will not guarantee a resultant healthy seed crop. It has been shown that contaminated and diseased seed may be freed from infection by hot-water treatment, whereas the use of organo-mercury disinfectants applied dry or by the short wet method were only partially effective. Treatment with hot water for 15 min. at 50° C. following a 4 hr. pre-immersion in tepid water, or for 30 min. at 50° C. without pre-immersion provided complete control, and the germination of the seed was in no case impaired when it was thoroughly dried immediately after treatment.

Introduction

It was in New Zealand in 1923 (Hyde, 1932), when the perennial rye-grass (*Lolium perenne*, L.) seed crops of the Sandon district were poor, that the question of low percentage germination of the seed first received serious attention. From then on weather records were kept, and it was stated by Foy (1927) that the low germination was attributable to wet conditions prevailing during harvest and that whereas rye-grass could not tolerate humid conditions during the final maturation of the seed, the reverse appeared to be the case for crested dog's-tail (*Cynosurus cristatus* L.) and Chewing's fescue (*Festuca rubra* L. var. *fallax* Thuill). An experiment carried out in 1929 (Hyde, 1932) showed the condition to be independent of harvesting methods. Kernels were then examined, and abnormal caryopses incapable of germination were discovered which carried both fungal mycelium and spores; it was then decided that the trouble might be due to a fungal parasite. The superior strains of rye-grass, the cultivation of which was being encouraged in New Zealand, were found to be more susceptible than inferior strains, and in 1938 Hyde (1938*a*) recorded severe infection of the seed of Italian rye-grass (*Lolium italicum* Braun). He states (Hyde, 1938*b*): 'As is now generally known, the low germination so common in perennial rye-grass crops is attributable in most instances to the attacks of *Pullularia*, a fungal parasite of the grass kernel.' The disease was fully investigated by Neill & Hyde (1939), who aptly named the trouble blind-seed disease and discovered the perfect stage of the responsible parasite which they placed tentatively in the genus *Helotium*. In Scotland, in the same year, Noble (1939) isolated *Pullularia pullulans* (de Bary) Berkhout from diseased seed, and Gemmell in 1940 published a bulletin entitled 'The disease of rye-grass seed attributed to *Pullularia pullulans*'.

In Northern Ireland the tendency for the germinating capacity of perennial rye-grass seed to be lower in some seasons than in others has been common knowledge for a long time, but it was not until the winter of 1939, when a sample of seed harvested from a pedigree strain (S 23) showed a very low germination, that the problem was investigated. On examination of the seed the symptoms of blind-seed disease were at once evident, and in the course of cultural work it soon became apparent that the blind-seed fungus and *P. pullulans* were not one and the same organism, although both were commonly found to be present on the same seed. This same observation has been made in Scotland (Wilson *et al.* 1940*a*). The perfect stage of the blind-seed fungus was found in Northern Ireland in the early summer of 1940 when infection experiments were carried out to investigate the pathogenicity of this fungus and *P. pullulans* (Muskett & Calvert, 1940). It was shown that, using both macroconidia (New Zealand and Northern Ireland isolations) and ascospores for inoculation purposes, the blind-seed fungus reacted as a parasite, whereas the conidia of *P. pullulans* produced no infection and the role of this fungus must, therefore, be regarded as saprophytic. In the same year Glasscock (1940) reported upon the occurrence of the disease in southern England. Gray (1942), working in Scotland, tentatively named the fungus *Phialea mucosa* n.sp., but Neill & Hyde (1942) regard it as synonymous with *P. temulenta* Prill. & Delacr. In the *List of Common British Plant Diseases* (Brit. Mycol. Soc. 1944) the causative agent of the disease is given as *Phialea temulenta* Prill. & Delacr., syn. *P. mucosa* E. G. Gray. More recently the fungus has been recorded in the United States of America (Fischer, 1944).

The present paper deals with an account of the work which has been carried out in Northern Ireland and which has been concerned in the main with a study of the life history of the parasite and an investigation of the possibility of controlling the disease by seed disinfection.

Seed examination and the isolation of the causal fungus

Infected samples of rye-grass seed cannot be distinguished from healthy samples by their external appearance. To examine seed samples for infection, each seed is placed in a drop of water on a microscope slide, and after one or both glumes have been removed it is examined microscopically, using a magnification of approximately × 100. The spores of *P. temulenta* are present in large numbers on infected caryopses and are easily observed as they float out into the water surrounding the seed.

Severely affected seeds are opaque when examined under the diaphanoscope (Hyde, 1932; Noble 1939). This method, however, is not satisfactory for use in making an accurate determination of percentage infection because the opacity decreases as the severity of the attack lessens, and it becomes increasingly difficult to distinguish between diseased and healthy seeds. For the separation of heavily infected seeds the method is useful and has been used largely during this investigation for obtaining small samples of such for experimental purposes. Occasionally opaque seeds were found which germinated normally and showed an absence of spores. In such cases the opacity was found to be due to pigmentation or weathering. Noble (1939) examined one sample of Danish seed in which the opaque seeds germinated to the extent of 93 %, and she attributed the opacity to weathering.

In January 1940 an attempt was made to evolve a method of seed examination similar to that used by Muskett & Malone (1941) for flax seed. Whole seeds

from several samples were plated out on a variety of media in Petri dishes and incubated for 5 days at 22° C., but owing to the vigorous growth of bacteria it was found necessary to acidify the media. Among the many fungi which produced mycelium was *Pullularia pullulans*, which at that time was generally supposed to be the organism responsible for blind-seed disease (Hyde, 1938*b*; Noble, 1939). Acid glucose agar was found to be the most suitable medium for the purpose, and the characteristic radial growth of *P. pullulans* was easily identified (Pl. 10, fig. 1). During the course of this work interesting results were obtained which inclined the writers to the view that *P. pullulans* was not the cause of the disease. In the first place the smaller, oval, non-guttulate and somewhat irregular spores produced by *P. pullulans* were quite unlike the regular allantoid spores found on the caryopses of diseased seeds. Quite frequently both types were present on the same seed. Secondly, there was no relation between the results obtained by the plating out method, assuming *P. pullulans* to be the parasite, and those obtained when the seed sample was examined microscopically. For instance, one sample gave 61% infection when plated out and 90% when examined microscopically, while another gave 80 and 6% respectively. Moreover, as the germination in the case of the second sample was over 90%, it seemed absurd that 80% of the seeds could have been infected. Further experiments in which whole seeds, glumes and caryopses were plated out separately showed that *P. pullulans* was present more often on the glumes than on the caryopses, and that it could be controlled by surface sterilization with 0·1% mercuric chloride.

On suspecting that *P. pullulans* was not the cause of the disease an attempt was made to obtain a culture of the blind-seed fungus from the allantoid spores present on infected seed. As it was now 6 months after harvest, some difficulty was experienced in getting any to germinate, but eventually a few viable specimens did germinate and these were grown on to produce single-spore cultures on 2% malt-extract agar. The mycelium grew very slowly (Pl. 10, fig. 1), but, after 7 days, sporing was observed and the culture began to take on a slimy appearance. These spores resembled those found on diseased seeds and were quite different from those produced by *P. pullulans* in culture. Germination took place in water overnight, whereas in the case of *P. pullulans* budding was observed to occur.

Neill & Hyde (1939) isolated the blind-seed fungus from diseased seeds after the seeds had been surface sterilized and bisected before being plated out on a nutrient medium. On receiving their publication the method was investigated. Glumes were removed from 100 seeds taken from a heavily infected sample, and the caryopses were surface sterilized by immersion in 0·1% mercuric chloride for 10 min. They were then washed in sterile water, bisected and plated out on 2% malt-extract agar. Within a week hyphae had grown from several and were producing spores. The fungus was slow growing and appeared to be similar in all respects to that produced by allantoid spores taken from seed. A culture was not obtained by plating out whole seeds, indicating that the mycelium is present in the internal tissues of the caryopsis.

Experiments were carried out to ascertain whether the bisection method could be used for the estimation of the percentage infection of rye-grass seed samples. This method would give the percentage of seeds in which the fungus is viable, whereas the microscopic examination of seed for spores does not take the viability of the organism into account. These experiments demonstrated that even with freshly harvested seed where the fungus would be viable in every case, the estimate of infection obtained by the cultural method was likely to be much lower than that given by microscopic examination. This discrepancy is probably explained in part by the killing of the fungus in some of the seeds resultant upon surface sterilization and also by its non-exposure in some cases following upon the bisection of the seed. It was therefore decided that the microscopic method is most suitable for the examination of seed samples for percentage infection with *P. temulenta*. Should information be desired regarding the viability of the parasite, this can be obtained by selecting a sample of heavily infected (opaque) seeds by the diaphanoscope and employing the cultural method.

Isolations of *P. temulenta* were made from apothecia. A suitable method used for obtaining cultures of this and other fungi from apothecia is by placing the apothecium hymenium, downwards, on the point of a sterile darning needle pushed through a cotton-wool plug and inserting it in a small conical flask containing a layer of medium. The spores are discharged, germinate, and in 2–3 days the fungus can be transferred to Petri dishes or slopes.

Growth of *Phialea temulenta* on media

On all media growth is slow and the mycelium is partly submerged. Macroconidium formation was observed by microscopic examination at intervals over a period with a magnification of approximately × 600, using a culture commencing to spore. Spores are produced from short conidiophores formed at intervals along the hypha. When the first spore is formed contact with the conidiophore is broken and a second developing pushes it aside (Text-fig. 1). This process continues until a cluster is formed with most of the macroconidia arranged perpendicularly to the hypha. The clusters eventually join and the hypha is surrounded with a mass of spores. The mucilage produced in culture and on infected caryopses covers and holds together the macroconidia,

thus giving the symmetrical cluster formation. The amount of mucilage seems to vary directly with spore production which is affected by the medium used. White aerial mycelium is produced on some media a few weeks after inoculation. On 2% malt-extract agar (Pl. 10, fig. 2) and potato-dextrose agar spores are produced in large numbers and, when grown on slopes, the conidial slime usually trickles down to the bottom of the tube. The colour varies from light to medium brown and some aerial mycelium is produced. The addition of 1·0% of peptone to these media increases spore and thus mucilage production, deepens the colour to chocolate brown, and when dried out the surface of the culture has a corrugated

Text-fig. 1. The formation of macroconidia by *Phialea temulenta* culture on plain agar.

appearance. A 2% Quaker-oat agar encourages sporing, and the production of aerial mycelium was greater than with any other medium used. On Czapek's medium the fungus has a rope-like branched appearance (Pl. 10, fig. 3) and turns chocolate brown after a few weeks. This effect is greatly accentuated by the addition of 1·0% of peptone (Pl. 10, fig. 4). The hyphae on prune agar are mostly submerged and the surface of the medium is not very slimy; little aerial mycelium is produced, but the colour of the medium darkens to a deep brown. On 2% glucose agar, 2% plain agar and Barnes's medium growth is sparse, spore production small and the cultures lack the normal slimy appearance. There is no aerial mycelium. The macroconidia produced in culture are not so regular in shape as those present on diseased seeds.

The life history of *Phialea temulenta*

The life history of *P. temulenta* was first elucidated by Neill & Hyde (1939) in New Zealand, and their findings have largely been confirmed in Northern Ireland. The fungus is carried over the winter in diseased seeds, the caryopses of which are rust brown in colour, shrivelled and rough on the surface. Healthy caryopses are normally golden brown, plump and of smooth appearance to the naked eye. Steeping the seed in water enables the colour difference between severely infected and healthy caryopses to be detected without removing the glumes. The fungal mycelium is present in the tissues of the caryopsis, and there is a thin layer of hyphae on the outside from which the numerous macroconidia are produced. The internal mycelium gives rise to the next two phases in the life history.

When infected seeds are sown they remain comparatively unchanged in the soil and do not decay as do other seeds which fail to germinate. In the late winter or early spring after sowing many give rise to pinkish, pulvinate, gelatinous sporodochia (0·5–1·0 × 0·4–0·6 mm.), which may be borne on any part of the caryopsis (Pl. 10, fig. 5). From a central core conidiophores arise in which numerous microconidia are produced endogenously (Wilson *et al.* 1940*b*). These spores are ovoid, unicellular, hyaline, with one gutta and measure 2·5–3·5 × 2·0–3·0 μ; germination has not been observed and their function is not definitely known.

Sporodochia were obtained in culture at room temperature, approximately 3 months after inoculation on 2% malt-extract agar, 2% Quaker-oat agar, and potato-dextrose peptone agar. Their production was encouraged when cultures were placed for a few weeks in the refrigerator at 5° C. (Pl. 11, figs. 1, 2). They were also produced in large numbers when conical flasks and Petri dishes containing cultures were buried in soil out of doors, during the winter.

The perfect stage of *P. temulenta* appears in early summer when rye-grass is in flower. The apothecia are found on the surface of the soil, having grown from diseased seeds, each of which may give rise to one or sometimes two or three (Pl. 10, fig. 6). In pot experiments less than 10% of infected non-germinating seeds produced apothecia, and the same is probably true in the field although, under suitable conditions, when heavily infected seed has been sown, the number present can be very great (Pl. 11, fig. 3).

Apothecia of many other discomycetes, found associated with rye-grass and other detritus in the field and often quite similar in appearance to those of *P. temulenta*, were collected. However, the cultures prepared from them showed a much wider variation in form than did the apothecia themselves, and only *P. temulenta* produced a mycelium which

spored readily. Among those collected were two species found on the basal leaf sheaths of living rye-grass plants, and one of these is believed to be the 'Number 2' fungus described by Neill & Hyde (1939) (Pl. 11, fig. 4).

The disk of the apothecium is at first cupulate, but with age it becomes saucer-shaped and then flat, while the colour changes from a light pinkish-brown to deep brown. The margin is smooth and entire and the diameter varies from 1·5 to 4·0 mm. The stipe, arising from the mycelium in the seed and composed of parallel hyphae 4·5–5·0 μ in diameter, varies in length from 1 to 10 mm. according to the depth of soil covering the seed and increases in diameter nearing the disk. In the hymenial layer the asci are interspersed with uniformly cylindrical, non-septate paraphyses not swollen at the apex. The asci are cylindrical clavate, 70–120 × 5·0–7·5 μ (mean 93 × 6·1 μ), with eight spores obliquely placed in a single row in the upper two-thirds and released by a plug which does not stain blue with iodine. The ascospores are hyaline, smooth, elliptical, somewhat pointed at the ends, usually with two refractive globules and measure 8·5–12·7 × 3·4–5·1 μ (mean 10·2 × 4·1 μ). They put out one or two germ tubes when germinating, and in the latter case a septum is usually formed. The asci ripen in succession so that the spores can be released over the whole flowering period of the grass. Slight atmospheric changes are sufficient to operate the discharge mechanism. In the laboratory visible puffs of spores were observed when the lids of Petri dishes, containing apothecia, were lifted.

In the field the ascospores are discharged and dispersed among the rye-grass plants by air movements. Primary infection takes place when a spore, coming in contact with a rye-grass flower, obtains suitable conditions for germination. Wilson *et al.* (1945) observed that in recently fertilized ovaries infection appeared to occur immediately below the base of the stigmata. The first external symptom of the disease is the presence, observed in inoculation experiments about 14 days after infection, of macroconidia on the caryopses which lose their normal unmatured green colour and take on a brownish tinge. In Scotland (Wilson *et al.* 1945) macroconidia were found to be present on the caryopses 9 days after infection. The macroconidia are produced in large numbers and are embedded in a slimy matrix which covers the caryopsis and very occasionally oozes out between the glumes. The spores are smooth, hyaline, thin-walled, allantoid in shape, biguttulate and measure 9·8–18·0 × 2·5–4·1 μ (mean 13·6 × 3·3 μ). When germinating they swell and put out one or occasionally two germ tubes.

Secondary infection in the field is brought about by the transference of macroconidia from infected seeds to flowers or lately fertilized ovaries on the same head or on the heads of adjoining plants. Since the spores are held in a mucilaginous matrix, which is soluble in water, dispersal can only take place by touch when the heads come in contact with each other or when the spores are carried in rain water or possibly by insects which may be attracted by the mucilage. Cool, moist weather conditions, as well as aiding dispersal, provide ideal conditions for infection due to the reduction in the rate at which pollination occurs.

Inoculation experiments have shown that the possibility of infection, using either ascospores or macroconidia, decreases rapidly after flowering has ceased. Since some further seed development takes place after infection and before growth is arrested, diseased seeds ranging from those with poorly developed caryopses to those with caryopses of normal size are produced. The former are removed to a large extent during the cleaning processes, and a high percentage of such could have a serious effect on the yield of dressed seed (Gemmell, 1940). In an infected caryopsis of normal size the embryo may not be killed by the fungus, and germination tests show that usually the number of seeds which do not germinate is less than the percentage of infected seeds estimated by microscopic examination. Neill & Hyde (1939) suggest that in 'blind seeds', which do not germinate, the embryo is kept in a state of suspended animation and that this may account for the fact that such seeds do not decay in the soil; this, however, may be due to the presence of the fungal mycelium, and it has been observed that 'blind seeds' do decay in the soil when the fungus has been killed by hot-water treatment (Calvert & Muskett, 1944). Although an infected seed may germinate, it is unlikely that such a seed will produce a normal plant and also an apothecium from the mycelium in its tissues since, in the production of a normal seedling, all the food material in the seed is utilized. During this investigation hundreds of apothecia have been collected but none growing from seeds with normal plants attached. On the other hand apothecia, shown by culturing to be those of *P. temulenta*, have been collected growing from seeds which had produced very diminutive plants (Pl. 11, fig. 5).

As the infected seed ripens, the mucilage on the caryopsis dries, forming a hard reddish brown crust in which the macroconidia are embedded. These spores are essentially thin-walled summer spores which spread the disease in the growing crop. Viability tests have been carried out with macroconidia from infected seeds taken from several samples harvested in the 4 years 1939–42 inclusive. The earliest and latest dates respectively on which spores from the various samples were last observed to germinate in drops of water were as follows:

1939	Earliest, not recorded	Latest, 5 Mar. 1940
1940	Earliest, 24 Oct. 1940	Latest, not recorded
1941	Earliest, 12 Nov. 1941	Latest, 4 Feb. 1942
1942	Earliest, 31 Dec. 1942	Latest, 31 Mar. 1943

There was considerable variation in viability between macroconidia from different seed samples and also between those from seeds in the same sample. Storage in a cool, dry basement as compared with storage in the laboratory tended to increase the period of viability by a few weeks. Generally it decreased rapidly in from 3 to 4 months after harvesting, and only in a few cases did spores remain viable after a storage period of 6 months. It thus seems certain that the disease is perpetuated by the viable mycelium in the caryopsis of the seed.

Pathogenicity of the fungus

Hyde (1932) found macroconidia of *P. temulenta* on seeds taken from samples of low germinating capacity, but he was not certain whether the fungus caused the disease or whether it was a saprophyte following upon the death of the seed by some physiological cause. Neill & Hyde (1939) proved it to be parasitic by dipping a few flowering heads of rye-grass in spore suspensions and obtaining typical blind-seed symptoms, using either macroconidia or ascospores.

Inoculation experiments were carried out in Northern Ireland during the summers of 1940 and 1941.

1940. Turves of commercial perennial rye-grass, in the pre-flowering stage, were brought into the laboratory greenhouse, and when in full flower a few heads from each were inoculated by immersion in spore suspensions in sterile water. This was done by dipping the heads in a 100 ml. measuring cylinder almost filled with a spore suspension and agitating them in the liquid for about a minute to ensure contact between the suspension and the flowers. The heads were then kept moist by placing them for 2 days in cylindrical glass museum jars lined with damp blotting paper and suspended from the roof of the greenhouse (Pl. 11, fig. 6). After removing them to the open air the turves were isolated and the seeds allowed to ripen normally. Suspensions of macrospores (Northern Ireland and New Zealand isolations), ascospores of *P. temulenta* and conidia of *P. pullulans*, together with sterile water as control were used in the experiment, and the results (Muskett & Calvert, 1940) showed that inoculation with macroconidia or ascospores of *P. temulenta* produced the typical symptoms of blind-seed disease, while with conidia of *P. pullulans* there was no perceptible damage and the fungus appeared to be saprophytic. There was insufficient seed available to carry out comprehensive germination tests, but those made indicated that infection with *P. temulenta* and low germination were correlated. When isolated from seeds which had been inoculated the fungus showed characteristic growth in culture.

Further inoculations were carried out 1, 2 and 3 weeks after flowering, using the same technique, and while a low percentage of less severe infection was obtained 1 week after flowering the results were negative in the other two cases, indicating that the possibility of infection is greatly reduced after fertilization has taken place.

1941. Experiments were carried out on a larger scale than in the previous year, using one commercial and two pedigree strains of perennial rye-grass and a commercial strain of Italian rye-grass. The same technique was employed with the difference that the heads were inoculated on two successive days in order to increase the amount of infection. Simultaneously, experiments were carried out in which the heads were left in the spore suspensions overnight before being placed in the moist chambers. The results from the two series were very similar. The following spore suspensions were used:

(1) Conidia of *P. pullulans* isolated in Northern Ireland from rye-grass seed.

(2) Macroconidia of *P. temulenta* isolated in Northern Ireland from rye-grass seed in 1940. (This suspension was used only for the first two experiments with commercial perennial rye-grass.)

(3) Macroconidia of *P. temulenta* isolated in Northern Ireland from rye-grass seed in 1941.

(4) Macroconidia of *P. temulenta* isolated in New Zealand by Neill.

(5) Ascospores from apothecia of *P. temulenta*. (These were not available for experiments with pedigree perennial rye-grass (S 23) and commercial Italian rye-grass carried out on 3 July.)

(6) Control: heads immersed in sterile water.

Macroconidia of *P. temulenta* were found 17 days after the first inoculation, on seeds from each of three groups of commercial perennial rye-grass inoculated with macrospore suspensions of that fungus. In the previous year 16 days elapsed before spores were seen. With pedigree perennial rye-grass (S 24) and Italian rye-grass spores were found after 15 and 13 days respectively. When ripe the seeds were removed from the heads and 200 from each group were examined for infection. Germination tests were carried out where 300 seeds were available. Table 1 shows the percentages of infection and of germination obtained from one series of experiments. In the case of some of the later inoculations flowering had commenced in the field before the turves were lifted, and this could account for the traces of infection occurring in the control and *Pullularia*-inoculated groups.

The results confirmed the findings of the 1940 experiments. Infection with *P. temulenta* was higher and germinations were affected to a marked degree. *P. pullulans* again caused no visible injury to the seed, and germination was not impaired so that its saprophytic character seemed to be certain. Only with commercial perennial rye-grass were inoculations carried out after flowering [the second inoculations on pedigree perennial rye-grass (S 23) and

Italian rye-grass having been performed when the grass was still in flower], and the results showed that susceptibility to infection was negligible when the seed had reached a certain stage of development.

RELATIVE SUSCEPTIBILITY OF RYE-GRASS STRAINS TO INFECTION WITH BLIND-SEED DISEASE

The results of the above experiments give little information on the varying susceptibility of the rye-grass, but Hyde (1938*a*) gives an instance where many crops of Italian rye-grass were severely affected and low germination resulted.

Flowering heads of commercial and pedigree perennial rye-grass and commercial Italian rye-grass were kept under observation during the summer of 1942 to ascertain if there was any significant difference in the length of time during which the flowers remained open. There were variations within each strain due to varying intensity of sunlight and

TABLE 1. *The percentages of seed infection with* P. temulenta, *and percentages of germination obtained from inoculation experiments* (1941)

(Heads agitated in spore suspension on two successive days)

	Commercial perennial rye-grass						Perennial rye-grass (S 24)	
Date of inoculation	20 June		2 July		15 July		26 June	
Spore suspensions	Infection	Germination	Infection	Germination	Infection	Germination	Infection	Germination
Control (sterile water)	0	97	0	96	0	95	30	93
Macroconidia of *P. temulenta* (isolated in Northern Ireland, 1940)	27	78	3	97	—	—	—	—
Macroconidia of *P. temulenta* (isolated in Northern Ireland, 1941)	49	69	5	96	1	95	76	79
Macroconidia of *P. temulenta* (isolated in New Zealand)	22	78	4	96	1	96	75	75
Ascospores of *P. temulenta*	9	96	0	97	0	96	69	76
Conidia of *P. pullulans*	0	96	1	98	0	97	18	94

	Perennial rye-grass (S 23)				Italian rye-grass			
Date of inoculation	27 June		3 July		28 June		3 July	
Spore suspensions	Infection	Germination	Infection	Germination	Infection	Germination	Infection	Germination
Control (sterile water)	1	95	4	93	0	96	2	96
Macroconidia of *P. temulenta* (isolated in Northern Ireland, 1940)	—	—	—	—	—	—	—	—
Macroconidia of *P. temulenta* (isolated in Northern Ireland, 1941)	80	62	65	84	87	58	60	57
Macroconidia of *P. temulenta* (isolated in New Zealand)	51	91	86	70	63	73	63	67
Ascospores of *P. temulenta*	32	81	—	—	20	94	—	—
Conidia of *P. pullulans*	2	—	24	—	3	95	2	96

strains employed to attack by *P. temulenta*. While the figures do indicate that commercial perennial rye-grass is not so liable to attack as the pedigree strains, there is no proof that Italian rye-grass is any more resistant. The evidence is that once the fungus has gained an entry into the open flower and obtained suitable conditions for germination an infected seed is likely to be produced. The examination of many samples has shown that in the field Italian and commercial perennial rye-grass are generally less susceptible than the pedigree strains of perennial

atmospheric humidity, but there was little difference between them in the average flowering time of individual flowers. On the other hand, the full flowering period of the crop may vary, and it was observed that pedigree perennial rye-grass (S 23), which was the most susceptible strain met with in Northern Ireland, had a prolonged and late-flowering period. At least 17 days after flowering had commenced in the field, fresh flowers were opening and consequently conidia from primarily infected seeds would have been available for secondary infection. The

interaction of three factors, namely, flowering of the grass, maximum ascospore dispersal and cool moist weather conditions, is important, and when all three are present simultaneously heavy infection is likely to occur. In 1939 such weather conditions in June resulted in a high percentage of infected seeds, especially in pedigree crops, in Northern Ireland, and when similar conditions prevailed in 1943 and 1944 severe attacks occurred as expected.

Relationship between seed infection and germinating capacity

The correlation between the percentage of infected seeds and the percentage germination was calculated from the data obtained from the inoculation experiments carried out in 1941. The amount of infection was not affected by the different techniques employed in the two series of experiments, and in calculating the correlation coefficient the values, obtained on the different occasions, have been considered together for each variety. The following results were obtained:

Commercial perennial rye-grass $r = -0{\cdot}9664$ ($n = 29$, 0·1 % probability value = 0·5627)

Pedigree perennial rye-grass (S 24) $r = -0{\cdot}8230$ ($n = 8$, 1 % probability value = 0·7646)

Pedigree perennial rye-grass (S 23) $r = -0{\cdot}7709$ ($n = 12$, 1 % probability value = 0·6614)

Italian rye-grass $r = -0{\cdot}9398$ ($n = 14$, 0·1 % probability value = 0·7420)

The correlation coefficients between the percentage of infected seed and of germination obtained for samples of S 24 perennial rye-grass received for examination in 1940, 1942 and 1943 were also calculated. The percentage germination varied from 90 to 98 in 1940, from 89 to 99 in 1942 and from 49 to 98 in 1943, whereas the range in the percentage of infected seeds in each of these years was 0–14, 0–11 and 0–82 respectively. The results obtained were as follows:

1940 $r = -0{\cdot}5784$ ($n = 18$, 1 % probability value = 0·5614),

1942 $r = -0{\cdot}4311$ ($n = 38$, 1 % probability value = 0·4032),

1943 $r = -0{\cdot}6937$ ($n = 157$, 0·1 % probability value = 0·3211 when $n = 100$).

In all cases the values obtained for the correlation coefficient are very significant and negative, indicating that the germination decreases as the percentage of infected seeds increases.

Host range of the parasite

During July and August 1942 heads of several grasses were collected in and around a field in which numerous apothecia of *P. temulenta* had developed from diseased seeds of perennial rye-grass. Seeds from each species were examined microscopically for the presence of macroconidia and, when these were found, attempts were made to isolate the fungus by the bisection method. Cultures were obtained from sheep's fescue (*Festuca ovina* L.), bent-grass (*Agrostis canina* L.), fiorin-grass (*Agrostis palustris* Huds.), smooth-stalked meadow-grass (*Poa pratensis* L.), Yorkshire fog (*Holcus lanatus* L.), and crested dog's-tail (*Cynosurus cristatus* L.). The spores on the caryopses were identical with those found on rye-grass seeds, and the cultures had the typical conidial slime. In the case of crested dog's-tail and Yorkshire fog there were few macroconidia and the caryopses showed no sign of injury, but with the other species the symptoms were typical. Seeds of sweet vernal-grass (*Anthoxanthum odoratum* L.), soft brome (*Bromus mollis* L.), cocksfoot (*Dactylis glomerata* L.), meadow foxtail (*Alopecurus pratensis* L.) and tufted hair-grass (*Aira caespitosa* L.) were also examined and, while a few macroconidia were present on some kernels of the first three named, the fungus was not isolated from the caryopses of any of these species.

Perennial rye-grass was successfully inoculated in 1943 with suspensions containing macroconidia of *P. temulenta* isolated from bent-grass, sheep's fescue and smooth-stalked meadow-grass, while meadow fescue (*Festuca elatior* L.) and rough-stalked meadow-grass (*Poa trivialis* L.) were found to be heavily infected when macroconidia from a perennial rye-grass isolation were used. In New Zealand the disease has been recorded on tall fescue (*Festuca arundinacea* Hackel), meadow fescue and Chewing's fescue (*Festuca rubra* L. var. *fallax* Thuill), while rye (*Secale cereale* L.) and darnel (*Lolium temulentum* L.) have been successfully inoculated (Neill & Hyde, 1942). In Oregon (U.S.A.) apothecia have been found on seeds of *Lolium perenne*, *L. temulentum* and *Hordeum gussoneanum*, while the conidial stage of *P. temulenta* has been found on the seeds of a wide range of species (Fischer, 1944).

Control of blind-seed disease

By breeding resistant strains

No evidence has been produced to indicate that *P. temulenta* can be systemic in rye-grass plants, and an experiment carried out in New Zealand, while not conclusive, supports the view that it is not systemic (Neill & Hyde, 1942). Therefore, it must be presumed that primary infection is brought about by ascospores released from apothecia. Any direct attempt at control must aim at preventing the formation of apothecia or the ascospores from reaching the flowers at the critical period. The presence of the mycelium in the internal tissues of the caryopsis, the life history of the fungus which involves a long resting period in the soil, and the fact that the disease is widespread, all suggest that the ideal method of control would be the introduction of resistant strains.

The recently introduced leafy strains tend to be more susceptible than the commercial types, and thus the breeding of resistant strains, having these desirable characters, is a problem of great economic importance.

By crop management

In New Zealand, Gorman (1940) carried out experiments on the control of the disease by crop management and obtained best results with early closed up crops and with those which had a thick undergrowth of a pedigree strain of white clover. Noble & Gray (1945) suggest that the attack can be minimized if the rye-grass crop is encouraged by using a stiff-strawed nurse crop, by the application of lime in the rotation where necessary and by substituting nitrochalk for ammonium sulphate as a top-dressing where blind-seed disease is suspected. The rotation of crops may have an effect, and one case in Northern Ireland was significant. In 1941 (Calvert & Muskett, 1944) the seed from one field of S 24 rye-grass sown with disease-free seed in the spring of 1940, showed infection ranging from 31 to 55% for six samples examined. In the seed from several other crops, produced from the same parent seed, the disease was absent or present only to a very small degree. On investigating the crop rotations it was discovered that in the former case rye-grass had followed a 4-year ley, while in all other cases the seed had been sown on land which had been in arable crops for from 3 to 4 years. It seems clear that the amount of infection had been influenced by previous cropping.

By the loss of viability of the parasite in stored seed

Neill & Hyde (1939) found that in general the mycelium of *P. temulenta* lost its viability after 20 months' storage, although in one case it was isolated after a longer period. In Northern Ireland tests on the viability of the mycelium were carried out. Twenty-five infected seeds from each of three samples, two harvested in 1939 and one in 1940, were plated out in November 1940 on 2% malt-extract agar in Petri dishes after surface sterilization and bisection. The fungus was isolated from only one seed in the case of the two 16 months old samples, while from the seed harvested in 1940 it was recovered from sixteen seeds. In another case the fungus was not isolated when 200 infected seeds were plated out 18 months after harvesting. It was noticed, during laboratory experiments in August 1942, that with untreated seed the fungus growing out from bisected kernels on medium was losing its viability. The seed had been harvested in July 1941 and was stored in a cool, dry basement. Two hundred diseased seeds, selected from the sample with the aid of the diaphanoscope, were plated out at approximately monthly intervals from September 1942 until March 1943 to ascertain the viability of the fungus. The results, together with those obtained in previous experiments, are given in Table 2. It was noticeable that as the viability decreased so also did the vitality of the mycelium which was still alive. With freshly harvested seed the hyphae grew out into the medium in 5–7 days, but after a year's storage 10–15 days were necessary using the same medium.

The dying out of the mycelium has also been observed in the number of sporodochia produced. In March 1942 sporodochia were found on twenty-three out of fifty diseased seeds placed in soil in pots 3 months after harvesting. Diseased seeds from the same bulk were put in pots a year later and sporodochia were produced on only one seed of the fifty used as compared with twenty-nine from seed of the current year. By delaying the sowing of seed, a percentage of which is infected by *P. temulenta*, until the second spring after harvesting, a period of approximately 21 months, the possibility of primary infection from the seed sown would be small.

Table 2

Number of months harvested	5	6	11	12	14	15	16	18	19	20
Percentage *P. temulenta* growing out from the seed	43	28	15	14	10·5	5·5	3·5	1·5	1·0	0·0

By seed disinfection

(*a*) *Field experiments.* Two experiments were laid down at the Agricultural Research Institute, Hillsborough, Co. Down, in September 1940. The seed used for the first was S 23 perennial rye-grass harvested in 1939; it was 65% infected with blind-seed disease and showed a germination of 52%. Lots of 250 g. were submitted to the following treatments:

Dry: 3 and 6 g. Ceresan (1875A); 3 and 6 g. Agrosan G; 3 and 6 g. Nomersan (RD 7846).

Short wet: 40 ml. 2% Ceresan (U 564); 40 ml. 4% Ceresan (U564); 40 ml. 8% Ceresan (U564).

Hot water A: treatment as recommended for the control of loose smut (*Ustilago nuda*) of barley (Brooks, 1928), i.e. steeped for 4 hr. in tepid water and then plunged into water held at 49° C. for 5 min. followed by 10 min. at 51° C.

Hot water B: as for A except that the time of steep at 51° C. was reduced to 5 min.

One lot of seed was left untreated to serve as a control.

For the second experiment commercial perennial rye-grass seed harvested in 1940 was used. The percentage infection was 14% and the germination 91%. Lots of 250 g. were submitted to the following treatments:

Dry: 6 g. Ceresan (1875A); 6 g. Agrosan G; 6 g. Nomersan (RD 7846).

Short wet: 40 ml. 4% Ceresan (U564).

Hot water: as for A above.

One lot of seed was left untreated to serve as a control.

In carrying out the dry treatments the required quantity of disinfectant was shaken with the seed for 5 min. in a stoppered Erlenmeyer flask. For the short wet treatment the required amount of liquid was sprayed on to the seed in an Erlenmeyer flask with an atomizer, while shaking, and the whole thoroughly mixed by further shaking for 5 min. After treatments with hot water the seed was spread out in a thin layer to dry.

In the field the seed bed was prepared as in normal farm practice. One size of plot, 4 × 2 yd., was used, and paths 2 yd. wide were left between the plots. They were arranged in blocks, each block consisting of a complete experimental series, the position of each plot within the block being chosen at random. The experiment was carried out in quadruplicate. The seed was sown from paper envelopes in drills made at 6 in. intervals. The sowing rate was 60 lb./acre for the low-germinating S 23 seed and 40 lb./acre for the commercial seed.

Owing to the rather late sowing (25 September), the plants were not very well established before winter set in, with the result that growth, during the spring and early summer of 1941, was not luxuriant, especially in the plots sown with S 23 seed. During June and early July a search was made for apothecia, one-half of each plot being used for making observations and the other left untouched for seed production. Apothecia of *P. temulenta* were not found in either experiment in plots sown with seed treated with hot water, but they were found in all plots sown with seed receiving the other treatments. It would appear that seed disinfectants are not effective in preventing the further development of the parasite, whereas hot water kills the mycelium at the time of treatment.

Seed was saved from the half-plots left for this purpose, and a representative sample from each plot was examined for infection. All the samples were found to be infected and, although the percentage infection of the S 23 seed varied from 29 to 58, no treatment gave any consistent reduction on the control. The samples of commercial seed showed less infection, the range being from 5 to 42% with a mean of 23·8%, as compared with 44·2% for the S 23. It seems clear that ascospores had been carried from plot to plot, so that in order to test treatments effectively in the field, adequate isolation of the plots must be provided.

In the spring of 1942 small plots, well isolated from rye-grass crops, were sown with infected S 24 perennial rye-grass seed subjected to a hot-water treatment, which had been shown by laboratory experiments to be completely effective in controlling the parasite. Similar plots were sown in other districts with untreated seed from the same bulk sample. This seed, which was 30% infected with *P. temulenta*, was harvested in 1941 and showed a germination of 80%. Samples of 200 seeds each from some of the plots were examined microscopically for infection. The results showed a wide range in percentage infection from both treated and untreated plots (Calvert & Muskett, 1944). From ten plots sown with untreated seed the percentage infection varied from 0 to 38 with a mean of 8, while from fifteen plots sown with treated seed the range was from 0 to 55 with a mean of 11%. This suggests that infection from a source other than the seed sown had occurred, and, since these plots were situated at some distance from rye-grass crops, it is probable that apothecia produced by infected seed from plants in hedgerows, untopped pastures and waste ground provided the source of infection. Such being the case, seed treatment cannot be expected to provide satisfactory control except under conditions of perfect isolation.

This view is also supported by the fact that the percentage infection of the seed sown does not always correspond with that shown by the seed from the resultant crop. For instance, in 1942 some fields were sown with seed which was approximately 30% infected. In 1943, a year of heavy infection, it was expected that the seed from these crops would be more severely infected than that from those raised from seed almost free from infection, but, on the average, a higher contamination rate was not recorded.

(*b*) *Laboratory experiments.* As *P. temulenta* can be isolated, if viable, from bisected surface-sterilized diseased seeds plated out on a nutrient medium, it has been found possible to test seed disinfectants for the control of blind-seed disease in a short time in the laboratory.

The results from field experiments indicated that hot-water treatment was more effective in controlling the fungus than dry or short wet methods of seed disinfection. Using seed harvested in 1941 (showing 30% infection) the following treatments were applied in October of that year, using 250 g. seed in each case:

Dry: 6 g. Ceresan (1875A).

Short wet: 40 ml. 8% solution of Ceresan (U564).

Hot-water treatment: 4 hr. pre-immersion in tepid water followed by 5 min. at 49° C. and 10 min. at 51° C.

From each treated lot 200 opaque seeds were

selected, bisected on sterilized glass slides, the scalpel being sterilized after bisecting each group of ten seeds, and plated on 2% malt-extract agar. This medium had been found to be satisfactory for this purpose and was used in all similar experiments. For the control, 200 untreated opaque seeds were surface sterilized by immersion in 0·1% mercuric chloride for 30 min., washed in sterile water, bisected and plated out while still damp. Observations made after 12 days were as follows:

Ceresan (1875A): growth of *P. temulenta* from 11·5% of the seeds; some control of bacteria and moulds.

Ceresan (U564): growth from 10% of the seeds; some control of bacteria and moulds.

Hot water treatment: no growth observed; colonies of bacteria produced around most seeds.

Control: Growth from 43% of the bisected seeds; surface sterilization had controlled moulds and bacteria.

Opaque seeds from the above lots were also sown in pots soon after treatment, and these were examined in April 1942. Sporodochia were found on diseased seeds from the control, Ceresan (1875A) and Ceresan (U564) treated samples, but none was present on the seed which had been treated with hot water. Normally, non-germinating seeds infected with *P. temulenta* do not decay in the soil, but it was observed that, after hot-water treatment, the seeds had rotted leaving only the glumes and seed coat, while in the case of the other treatments they were quite firm and no rotting had taken place. This was again observed in 1943.

Treatment with hot water did not control the growth of all bacteria and moulds present on the seed, and an attempt was made to achieve this by using dilute solutions of Ceresan (U564) and mercuric chloride instead of hot water alone. Five concentrations of Ceresan (U564), ranging from 0·1 to 1·0%, and 0·1% of mercuric chloride were used, the treatments being carried out in the normal way. Concentrations of Ceresan (U564) above 0·2% and of 0·1% mercuric chloride gave complete control of moulds and bacteria, when the temperature of the solutions was 50° C., but it was found that the germination of the seed had been impaired. When the temperature was reduced to 40° C. *P. temulenta* was not fully controlled. No further experiments were carried out, but Ceresan (1875A), at the rate of 6 oz./bushel, was used as a dry-seed dressing after hot-water treatment, and was most effective in controlling the remaining moulds and bacteria. When applied without the hot-water treatment it was not nearly so effective. Germination tests showed that the value of Ceresan (1875A) following hot-water treatment is offset by the fact that there is a tendency for the germination to be impaired: this is probably due to the increased moisture content of the seed following steeping in hot water.

In July 1942 a series of experiments was undertaken in order to determine the optimum time and temperature required for immersion in hot water and whether pre-immersion in tepid water was necessary. The technique was similar to that already described, except that after treatment the seeds to be plated were surface sterilized in 0·1% mercuric chloride for 30 min. and washed in sterile water to prevent the growth of *P. temulenta* being masked by bacteria. S24 perennial rye-grass seed, harvested in 1941, was used until the fungus tended to lose viability in this particular sample, when S23 perennial rye-grass seed, harvested in 1942, was substituted.

When the temperature was kept constant at 50° C. and the time of steeping varied, the percentage of viable *P. temulenta* was found to be 13·0 for 5 min., 6·5 for 10 min., 1·0 for 15 min., and 0·5 for 20 min., the control registering 14%. With the time of steep kept constant at 15 min. and the temperature varied, the percentages were 15·5 at 40° C., 11·5 at 45° C. and 2·0 at 50° C. In neither case was the seed pre-immersed in tepid water, and it is significant that a 15 min. steep at 50° C. did not give complete control without previous immersion. In all earlier experiments the fungus had not been isolated when immersion in tepid water was followed by steeping for 5 min. at 49° C. and then for 10 min. at 51° C.

TABLE 3. *The effect of pre-immersion in tepid water*

	No pre-immersion				4 hr. pre-immersion		
Duration of treatment at 45° C. (min.)	0 (control)	15	20	30	15	20	30
% *P. temulenta* isolated from 1941 seed	—	3·0	2·5	0·0	0·5	0·0	0·0
% *P. temulenta* isolated from 1942 seed	26·5	23·0	11·5	2·0	5·0	4·5	0·0

The advantageous effect of pre-immersion was shown by treatments with the temperature reduced to 45° C. using seed harvested in 1941 and 1942 (Table 3). At this lower temperature the effect was accentuated. With seed harvested in 1942 and treatments at 50° C. lasting 15, 20, 30, 45 and 60 min., pre-immersion was compared with no pre-immersion. The fungus was isolated from 5·5 and 2·0% of the seeds plated from treatments of 15 and 20 min. duration without pre-immersion respectively, while all others gave complete control. Some reduction in germination occurred with 45 and 60 min. steeps, especially after pre-immersion.

An experiment was carried out in December 1942

to obtain information on the maximum temperature at which seed could be steeped, for up to 30 min., without damage to the germinating capacity. The treatments, without pre-immersion, were for 15 and 30 min. at temperatures of 50 and 55° C. respectively, and seed harvested in 1941 and 1942 was used. Diseased seeds from each treatment, with 1942 harvested seed, were tested for viability of *P. temulenta*, and Table 4 shows the results, together with the percentage germination of seed from all treatments. At 55° C. there was a definite reduction in germination, especially with the 30 min. steep, but at 50° C. the germination was unimpaired.

The conclusion drawn from the foregoing and other experiments is that the most promising seed treatments for the control of *P. temulenta* are: (*a*) pre-immersion for 4 hr. in tepid water followed by 15 min. in hot water at 50° C.; (*b*) 30 min. in hot water at 50° C., without pre-immersion. Both treatments have consistently given complete control. From the practical aspect the latter is more economical in that a pre-immersion in tepid water is not required.

(*c*) *Germination of hot-water-treated seed after storage.* The effect of storage on the germinating capacity of seed after hot-water treatment was investigated using S 24 rye-grass seed harvested in 1941 and 30% infected with *P. temulenta*. Treatments carried out in October 1941 and in May 1942 were as follows:

A. October 1941: 4 hr. pre-immersion in tepid water followed by 5 min. at 49° C. and 10 min. at 51° C.; dried and stored in a paper bag in the laboratory.

B. October 1941: 4 hr. pre-immersion in tepid water followed by 5 min. at 38° C. and 10 min. at 40° C.; dried and stored in a paper bag in the laboratory.

C. May 1942: 4 hr. pre-immersion in tepid water followed by 5 min. at 49° C. and 10 min. at 51° C.; dried and stored in a linen bag in a cool, dry basement.

D. Untreated: stored in a linen bag in a cool, dry basement.

Germination tests carried out at intervals (Table 5) showed little damage due to treatment, and suggest that if quickly and thoroughly dried, seed could be stored for at least 6 months after treatment without deterioration.

Further germination tests were carried out in March 1943 on the samples treated for 15 min. at 50° C. following pre-immersion and for 30 min. at 50° C. without pre-immersion, from July to December 1942, and stored in the laboratory. From the germination results obtained, as compared with those obtained before and immediately after treatment, no apparent deterioration had occurred since the time of treatment.

TABLE 4. *The effect on germination of hot-water treatment at* 50 *and* 55° C.

Treatment	1942 harvested seed		1941 harvested seed
	% *P. temulenta* isolated	% germination	% germination
Untreated	26·0	69	79
15 min. at 50° C.	6·0	71	79
30 min. at 50° C.	0·0	76	75
15 min. at 55° C.	0·0	68	71
30 min. at 55° C.	0·0	49	26

TABLE 5. *The percentage germination of rye-grass seed during storage, after hot-water treatment*

Treatment	Date of test and percentage germination					
	20. vi. 42	22. vii. 42	19. viii. 42	23. x. 42	22. 12. 42	23. ii. 43
A	80	80	83	77	78	75
B	78	82	80	76	73	77
C	85	80	84	76	71	85
D	81	81	81	77	85	85

Discussion

Blind-seed disease of rye-grass has been proved, by the isolation of the fungus and by inoculation experiments, to be due to the attack of a parasitic fungus [the 'blind-seed fungus' of Neill & Hyde (1939)], tentatively named *Phialea mucosa* n.sp. (Gray, 1942) and later shown to be synonymous with *P. temulenta* Prill. & Delacr. (Neill & Hyde, 1942). *Pullularia pullulans*, which is often present on rye-grass seed and was suggested by earlier investigators to be the causal parasite, has been shown to be saprophytic and does not reduce the germinating capacity of the seed. On media *P. pullulans* grows quickly with characteristic radial growth, the hyphae producing slimy radula spores while *Phialea temulenta* grows very slowly and macroconidia are produced at intervals along the hyphae from short conidiophores. On most media sporing takes place freely, and the mucilage produced covers the surface of the culture.

Several methods were employed in working out a technique for the estimation of infection in a sample of rye-grass seed, including the selection of opaque seeds by the use of the diaphanoscope and the plating out on media of whole and bisected seeds. Neither of these two methods gave an accurate estimate of infection, but the diaphanoscope has been used for the purpose of selecting small quantities of infected seed for experimental purposes, while the growing of the fungus from surface-sterilized bisected seeds on media has been most useful, especially in experiments on control in which the determination of the viability of the fungus was involved. The most satisfactory technique for the estimation of infection is to examine the seeds individually in drops of water under the microscope for the presence of macrospores, after the removal of one or both glumes. At best the method is tedious, but with practice it can be performed quite speedily.

The life history of the causal organism as worked out by Neill & Hyde (1939) has largely been confirmed in Northern Ireland. *P. temulenta* lives through the winter as mycelium in infected seeds; in early spring microconidia are produced endogenously from sporodochia arising from diseased kernels in the soil. The function of the microconidia is not known and their germination has not been observed.

Ascospores from apothecia on diseased seeds cause primary infection when rye-grass commences to flower. Wilson *et al.* (1945) state: 'In recently fertilized ovaries in which the nucellus has undergone only slight elongation infection by *P. mucosa* appears to take place immediately under the base of the stigmata.' During the present investigation the first symptom observed was the presence of macroconidia on the young caryopsis. These spores, which cause secondary infection, are thin-walled and have generally been found to lose viability in less than 6 months after harvesting. The presence of mycelium in the inner tissues inhibits the normal formation of embryo and endosperm which are developed to varying degrees according to the severity of infection and the stage at which it has taken place. Neill & Hyde (1939) state: 'Seeds infected during the early stages of growth fail to develop or become thin and light in weight and so are likely to be removed from the threshed sample during dressing. When, however, secondary infection takes place shortly before ripening, size and weight are unaffected and such seeds remain in the dressed sample.' It is the opinion of the writers that a high percentage of primarily infected seeds will be removed during dressing, and that secondary infection must take place not long after fertilization if blind seeds are to be produced. This deduction is based on the fact that, while the percentage of infected seeds increases from the first appearance of macroconidia until the seed ripens, there is a lag of 9–14 days between infection and the production of macroconidia. Also, in inoculation experiments, the percentage of infection dropped rapidly and proved to be of a superficial nature after flowering had ceased. In some cases infected seeds will germinate, but if germination does result in the production of a normal plant, it is unlikely that the fungus will develop to produce an apothecium.

The varying susceptibility of the different strains of perennial and Italian rye-grass to attack by the fungus requires further elucidation. The examination of many samples from crops has shown that normally commercial perennial and Italian rye-grass are less susceptible than the pedigree strains of perennial rye-grass. Whether the actual cause of this variation is due to inherent resistance characters in the grass or to factors which may vary from year to year is not fully known, but probably both are operative. Weather conditions, during and immediately after flowering, play an important part, and when cool moist conditions prevail heavy infection is likely to occur. The period of maximum ascospore dispersal will also have an effect, and Gemmell (1940), in correlating susceptibility with lateness of flowering, mentions this point. The simultaneous occurrence of these factors at flowering time may account for the heavy infection of several crops of Italian rye-grass recorded by Hyde (1938*a*). Observations on plants of various strains while flowering showed little difference in the average time which individual flowers remained open, but, in the field, it was noticed that S23 perennial rye-grass had a longer and later flowering period than commercial perennial rye-grass.

The correlation between percentage infection and percentage germination was calculated using the results from inoculation experiments carried out in 1941 and from crop samples examined in 1940, 1942 and 1943. In all cases the correlation coefficient was highly significant and negative, indicating that infection with *P. temulenta* reduces germination.

It is now generally recognized that *P. temulenta* is as widely distributed as rye-grass itself. As yet, little is known regarding the host range of this parasite, but it is found in the seeds of several other grasses. In Northern Ireland the fungus has been isolated from sheep's fescue, bent-grass, fiorin-grass, smooth-stalked meadow-grass, Yorkshire fog and crested dog's-tail. These grasses were collected in and around a field in which many apothecia were produced from 'blind seeds' of perennial rye-grass, and these may have been the source of infection. No information was obtained on how the presence of the fungus affected the germination of seeds of these species. Meadow fescue and rough-stalked meadow-grass were successfully inoculated with macroconidia of the fungus in 1943. In New Zealand (Neill & Hyde, 1942) the macroconidia of *P. temulenta* have been found on seeds of tall fescue, meadow

fescue and Chewing's fescue, while apothecia have been found on fallen seeds of tall fescue. Also, rye and darnel have been successfully inoculated and apothecia have been produced from infected seeds. In America it has been found on a variety of grasses (Fischer, 1944).

The control of blind-seed disease is a problem of economic importance, particularly as the newly introduced strains of perennial rye-grass are more susceptible to the disease. Because of their lower seed yields and, in some years, poorer germinating quality, farmers engaged in seed production may be inclined to continue growing commercial types, and this, in turn, would reduce the quantity available for the production of better pastures.

If seed is not sown until the second spring after harvesting, *P. temulenta* will generally have lost its viability and there will be little danger of infection from the seed sown. There are economic reasons for not practising this method of control, especially with seed of new strains which is not available in large quantities. Dry and short wet methods of seed disinfection in both field and laboratory experiments were not effective in controlling the fungus. The position of the mycelium in the inner tissues and the long resting period required before sporodochia and apothecia are produced suggest that effective treatment must kill the mycelium directly. For this reason the hot-water treatment, as recommended for loose smut of barley (Brooks, 1928), was tried and found to be effective in controlling the fungus without damaging the seed. Further work showed that the treatment could be simplified by dispensing with the pre-immersion steep and treating for 30 min. at 50° C. When the temperature was reduced to 45° C. the control of the fungus was not complete, while at higher temperatures than 50° C. the germination of the seed was impaired. After a storage period of more than a year the germinating capacity of seed treated at 50° C. or below had not deteriorated, and it is believed that, provided the seed is dried thoroughly immediately after treatment, there is little danger of reduction in germination during storage for a period of up to 6 months.

In the field no apothecia were found in plots sown with hot-water treated seed, but the seed produced was as heavily infected as the samples from surrounding plots, due to the dispersal of ascospores. When plots were sown in isolation from rye-grass crops there was little difference in the average percentage infection from plots sown with treated and untreated seed, although laboratory tests had shown that the treatment had killed the mycelium in the seed. It is thought that the source of infection was ascospores from apothecia on seed from plants growing in waste ground, hedgerows or pastures in proximity to the plots. Other evidence, such as the heavy infection of crops grown from practically disease-free seed and the heavy infection of a crop resulting from the ploughing up of a pasture and immediate resowing, suggest that the disease is widespread, and that although disease-free seed is sown infection is likely to occur if conditions are suitable at flowering time. This being so, the effective treatment of seed is no guarantee that the resultant crop will be disease free. Crop management may assist to some extent, but the breeding or selection of resistant strains seems to offer the best solution to the problem.

Since rye-grass is of primary importance as a forage crop, and as the use of the seed is largely limited to the propagation of the species, too much importance could be placed upon reduced germination due to the incidence of blind-seed disease. No evidence has been produced to show that the causal parasite has any effect on the crop other than the reduction in the germination of the seed. The pedigree strains are certainly more susceptible to heavy infection than commercial types, but if, on the other hand, the leafy growth of these strains makes them more valuable for pasture, then a certain reduction in the percentage germination of the seed would appear to be of secondary importance. In Northern Ireland there would be few seasons when, by proper blending, the seed of these new strains could not be brought up to a standard of germination suitable for incorporation in mixtures intended for pasture establishment in non-seed-producing areas. The best seed should be reserved for distribution in seed-producing areas; it is possible that some districts would, on the whole, be more suitable for the production of seed of certain strains than others, and, if so, the growth of such strains should be encouraged there instead of commercial types. By these means a supply of seed of valuable strains already in existence would be made available until such time as strains of equal value and resistant to blind-seed disease can be introduced.

The writers acknowledge the assistance of Mr J. P. Malone in the preparation of the photographs used in this paper. The help provided by the Seed Testing Station in carrying out a large number of germination tests and in supplying samples for examination for infection with *P. temulenta* is gratefully acknowledged, as also is the provision of facilities for field experiments provided by the Agricultural Research Institute, Hillsborough, Co. Down.

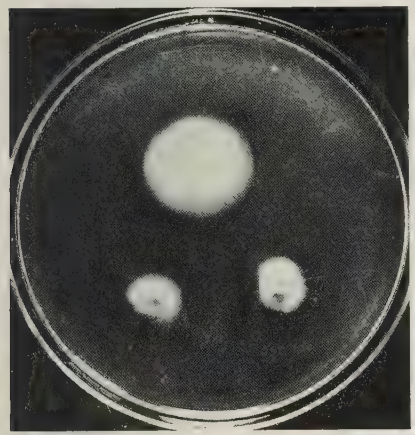


Fig. 1

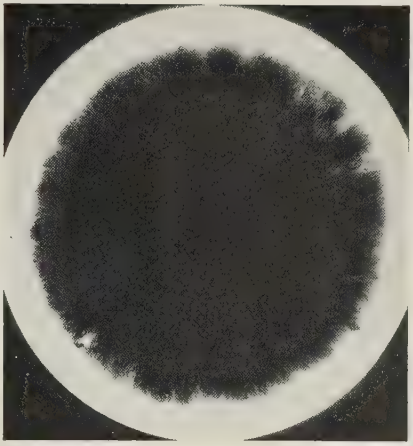


Fig. 2

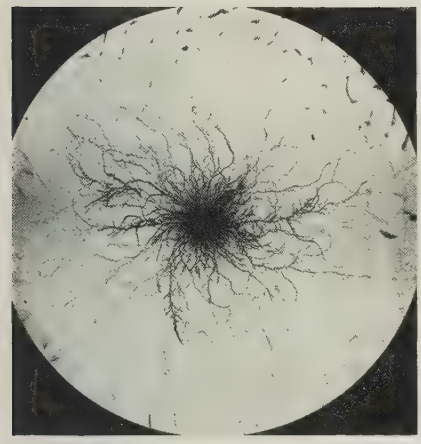


Fig. 3

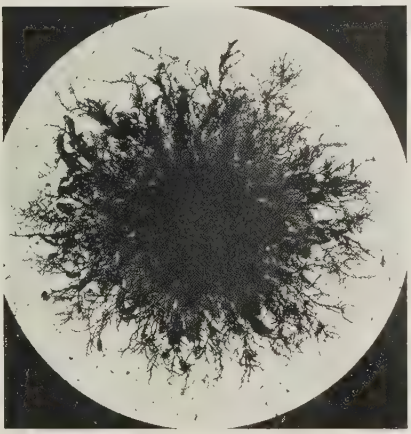


Fig. 4

Fig. 5

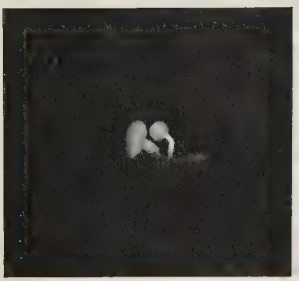


Fig. 6

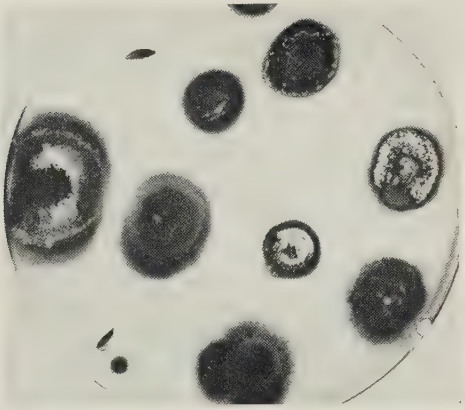


Fig. 1

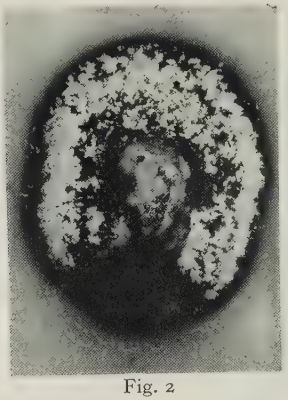


Fig. 2

Fig. 3

Fig. 5

Fig. 4

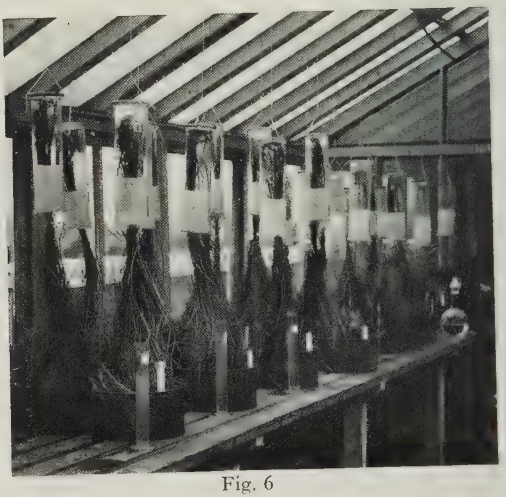


Fig. 6

REFERENCES

British Mycological Society (Plant Pathology Committee) (1944). *List of Common British Plant Diseases*. Camb. Univ. Press.

Brooks, F. T. (1928). *Plant Diseases*, p. 217. Oxford Univ. Press.

Calvert, E. L. & Muskett, A. E. (1944). Blind-seed disease of rye-grass. *Nature, Lond.*, **153**, 287.

Fischer, G. W. (1944). The blind-seed disease of rye-grass (*Lolium* spp.) in Oregon. Abstr. *Phytopathology*, **34**, 934.

Foy, N. R. (1927). The official seed-testing station (record of operations for 1926). *N.Z.J. Agric.*, **34**, 188.

Gemmell, A. R. (1940). The disease of rye-grass seed attributed to *Pullularia pullulans*. *Bull. W. Scot. Agric. Coll.*, no. 136, 16 pp.

Glasscock, H. H. (1940). Blind-seed disease of rye-grass. *Nature, Lond.*, **146**, 368.

Gorman, L. W. (1940). Blind-seed disease investigations. *N.Z.J. Sci. Tech.* A, **22**, 79. (Abstr. in *Rev. appl. Mycol.* **20**, 263.)

Gray, E. G. (1942). *Phialea mucosa* sp.nov., The blind-seed fungus. *Trans. Brit. mycol. Soc.* **25**, 329.

Hyde, E. O. C. (1932). Germinating capacity of perennial rye-grass seed. *N.Z.J. Agric.* **44**, 316.

—— (1938*a*). Germinating capacity of Italian rye-grass seed. *N.Z.J. Agric.* **56**, 357.

Hyde, E. O. C. (1938*b*). Detecting *Pullularia* infection in rye-grass seed crops. *N.Z.J. Agric.* **57**, 301.

Muskett, A. E. & Calvert, E. L. (1940). Blind-seed disease of rye-grass. *Nature, Lond.*, **146**, 200.

Muskett, A. E. & Malone, J. P. (1941). The Ulster method for the examination of flax seed for the presence of seed-borne parasites. *Ann. appl. Biol.* **28**, 8.

Neill, J. C. & Hyde, E. O. C. (1939). Blind-seed disease of rye-grass. *N.Z.J. Sci. Tech.* A, **20**, 281.

—— (1942). Blind-seed disease of rye-grass. II. *N.Z.J. Sci. Tech.* A, **24**, 65.

Noble, M. (1939). Notes on *Pullularia pullulans* in rye-grass seed and seed-testing methods as affecting detection of certain seed-borne diseases. *Ann. appl. Biol.* **26**, 630.

Noble, M. & Gray, E. G. (1945). Blind-seed disease of rye-grass. *Scot. J. Agric.* **25**, 94.

Wilson, M., Noble, M. & Gray, E. G. (1940*a*). Blind-seed disease of rye-grass. *Nature, Lond.*, **145**, 783.

—— —— —— (1940*b*). Blind-seed disease of rye-grass. *Nature, Lond.*, **146**, 492.

—— —— —— (1945). The blind-seed disease of rye-grass and its causal fungus. *Trans. roy Soc. Edinb.* **61**, 327.

EXPLANATION OF PLATES 10 AND 11

Plate 10

Fig. 1. One colony of *P. pullulans* (5 days old) and two colonies of *P. temulenta* (12 days old) on 2 % malt-extract agar.

Fig. 2. Growth of *P. temulenta* on 2 % malt-extract agar after 4 weeks' incubation at 22° C.

Fig. 3. Growth of *P. temulenta* on Czapek's medium after 4 weeks' incubation at 22° C.

Fig. 4. Growth of *P. temulenta* on Czapek's medium plus 1 % peptone after 4 weeks' incubation at 22° C.

Fig. 5. Sporodochia of *P. temulenta* on kernels of infected rye-grass seeds.

Fig. 6. Rye-grass seed bearing two apothecia of *P. temulenta*.

Plate 11

Fig. 1. Sporodochia produced from cultures of *P. temulenta* growing out from bisected seeds plated out on 2 % malt-extract agar.

Fig. 2. Enlargement of one of the colonies shown in fig. 1.

Fig. 3. Apothecia of *P. temulenta* on a small turf taken from a field which produced a heavily infected seed crop.

Fig. 4. Apothecia, believed to be those of the 'Number 2' fungus described by Neill & Hyde, produced on the basal leaf sheath of a living rye-grass plant.

Fig. 5. Apothecia of *P. temulenta* growing from seeds which had produced diminutive plants.

Fig. 6. Photograph illustrating the technique employed in inoculation experiments.

(*Received* 5 *May* 1945)

The incidence and distribution of some diseases of the adult honeybee (*Apis mellifera* L.) in England and Wales

By C. G. Butler, *Bee Department, Rothamsted Experimental Station, Harpenden, Herts*

(With 4 Text-figures)

Although epidemics of bee disease have been reported in Europe for hundreds of years it was not until recent times that detailed notes on the incidence and distribution of one of these epidemics, the so-called 'Isle of Wight disease' epidemic, were made.

Since 1918 four complaints of the adult honeybee, acarine disease, Nosema disease, Amoeba disease and bee paralysis, have been recognized in this country.

Surveys in England and Wales to determine the incidence and distribution of acarine disease, Nosema disease and Amoeba disease were carried out during the winters of 1941–2 and 1943–4. These surveys showed that none of these diseases is clearly confined to any definite areas of England and Wales. Acarine disease is, however, apparently more abundant in the west and south-west; and Nosema disease appears to be most prevalent in Buckinghamshire, Hertfordshire, Middlesex and Surrey, and also has other areas of heavy infection in Leicestershire and Cumberland, Lancashire and Yorkshire.

Sufficient cases of Amoeba disease have not yet been recorded for it to be possible to conclude that any one area is more heavily infected than another. It is estimated that approximately one out of every five or six of the colonies of bees in England and Wales is suffering from acarine disease. Less than 1 % are likely to be suffering from Nosema or Amoeba disease. Bee paralysis is both common and widespread, but probably does little serious harm.

Introduction

Records show that for hundreds of years periodical outbreaks of disease on an epidemic scale have decimated colonies of honeybees in this and other European countries. In the Court Rolls of the Manor of Heacham, in Norfolk, under the date 1372 and adjacent years, it is stated that numerous hives of bees were attacked by the murrain which was then raging amongst the cattle (Fraser, 1932). In some of the cases described in these old records, the external signs of the epidemic complaints are very similar to those which occur when a colony is infected with certain of the adult bee diseases known to-day.

It was not until recent times that detailed notes on the occurrence and distribution of one of these periodic epidemics in this country were recorded. In 1905, however, an epidemic of adult-bee disease attracted attention in the Isle of Wight and, according to Herrod-Hempsall (1937), spread rapidly throughout the bees of the island during the course of the next three years, eventually appearing on the mainland in 1909 in those counties nearest to the island, viz. Dorset, Hampshire, Surrey and Sussex. It then spread, according to the above author, to Cornwall, Glamorgan, Herefordshire and the western borders of Worcestershire and Yorkshire, as well as to Scotland and, later, Ireland. Possibly the importation of colonies of infected bees into these areas from those counties in which the epidemic had been observed in 1909 was responsible. Possibly, though it seems less probable on account of the widespread nature of the new outbreak centres, the local conditions favoured the development of the epidemic in these areas in which the disease or diseases concerned may have already been present in a mild form. From these centres of heavy infection the epidemic which had, on account of its supposed origin and lack of knowledge of the causative agent or agents, become known as the 'Isle of Wight disease', gradually spread in all directions, until by 1918 there was not a beekeeping district in Great Britain free from the epidemic. By means of a survey the Board of Agriculture estimated that the loss of colonies of bees in this country during 1918 reached the appalling figure of 90 %. In 1919, therefore, it was decided to establish a restocking scheme and, since data obtained from the 1918 survey had apparently tended to show that the Italian bee was less susceptible to 'Isle of Wight disease' than the British brown or black bee, to provide intending beekeepers in this country with colonies headed by Italian queens.

This very serious epidemic of 'Isle of Wight disease' greatly stimulated research on adult-bee diseases in this country and, in 1919, the causal agent of acarine disease, the mite *Acarapis woodi* (Rennie), was discovered by Dr Rennie of Aberdeen. This parasite is now believed by many workers to have been the prime, if not the only, agent responsible for the epidemic of 'Isle of Wight disease'.

In 1913 a second disease of adult bees, Nosema disease, caused by the protozoan *Nosema apis* (Zander), was recognized in this country. A third disease, Amoeba disease, caused by infestation of the Malphigian tubules of the adult bee by the protozoan

Malphighaemoeba mellificae (Prell), was recognized in 1929 by Morison (1931).

It is with the incidence and present distribution of these three diseases of the adult honeybee, acarine disease, Nosema disease and Amoeba disease, that the present paper is concerned.

Apparently no further survey of adult-bee diseases was carried out in this country until the winter 1941–2, when a survey was organized in England and Wales from the Bee Department, Rothamsted Experimental Station, with the approval and assistance of the Ministry of Agriculture and Fisheries. A similar survey was completed during the winter 1943–4 and, in addition to the samples of bees received for examination in connexion with these surveys, some 3500 samples of adult bees were sent to Rothamsted for examination for diseases during the period January 1941–October 1944. In this way a considerable amount of data on the incidence, distribution and seasonal periodicity of these diseases has been collected.

Method of conducting adult-bee disease surveys, 1941–2 and 1943–4

In the absence of any complete register of beekeepers, it was impracticable to make surveys which would cover all beekeepers in England and Wales. Probably about one-third of all beekeepers are members of Beekeepers' Associations, and the surveys which were carried out were necessarily based on members of Associations only. This may, of course, result in some bias towards the keener and better beekeepers, but it is not thought that this effect is very great.

Lists of members' names and addresses were obtained from each County Beekeepers' Association, and a letter was addressed to every twentieth name on these lists. This letter asked the recipient to send to Rothamsted a sample of 20–30 bees from a single colony, this colony being chosen at random (by the process of drawing lots) from all those in his possession. Beekeepers with more than ten colonies were asked to send two such samples.

By emphasizing the fact that the Rothamsted Bee Department was always willing to examine samples of bees suspected of disease, it was hoped to avoid any conscious or unconscious departure from random selection of the colonies used for the surveys.

In each of the surveys over 1000 beekeepers in England and Wales were approached and samples were received from about half of these persons. The number of samples obtained for the purposes of these surveys was planned only with the object of being able to discover any large and clearly defined geographical trends.

It is, of course, desirable to know the largest percentage of diseased bees in a large population which is likely to remain undetected in random samples of various sizes taken from this population. This has been calculated by Mr D. J. Finney of the Statistical Department, Rothamsted Experimental Station, and the results are shown in Table 1.

Table 1. *The largest percentage of diseased bees in a large population which is likely to remain undetected in a random sample of* n *bees taken from this population (at three levels of fiducial probability)*

n	$P=0{\cdot}1$	$P=0{\cdot}025$	$P=0{\cdot}005$
1	90·0	97·5	99·5
2	68·4	84·5	92·9
3	53·6	70·8	82·9
4	43·8	60·2	73·4
5	36·9	52·2	65·3
10	20·6	30·8	41·1
15	14·2	21·8	29·7
20	10·9	16·8	23·3
30	7·4	11·6	16·2
50	4·5	7·1	10·0
100	2·3	3·6	5·2

It will be seen from Table 1 that if the true percentage of infected bees within a colony of, for example, 30,000 bees, is 7·4%, then there is only a 10% probability of there being no infected bees in a sample of thirty; similarly, if the true percentage is 11·6%, there is only a probability of 2·5% of there being none infected in thirty, and for a true percentage of 16·2% the probability is 0·5%. Obviously, therefore, reasonably accurate results could be expected when samples each consisting of between twenty and thirty bees were examined, and it is clear that in order to attain any considerably greater degree of accuracy very much larger samples would have to be examined.

Results

(i) *Acarine disease*

The possibility of classifying the samples received for the 1941–2 and 1943–4 surveys, as clean, slightly infected, or seriously infected in respect of acarine disease was considered, but eventually it was decided to rely on the simple classification of clean or diseased for each county (in a few instances two counties were combined as being covered by a single Association—e.g. Leicestershire and Rutland) and a map (Fig. 1) has been prepared to show these various proportions. In all those counties from which less than twenty-five samples were received during the course of the two surveys a ? has been drawn to indicate that less reliability should be placed on the figures obtained for these counties than on those obtained from the majority of counties. In constructing Fig. 1 the whole country has been subdivided into four different levels of disease incidence; the grouping of neighbouring counties had to be somewhat arbitrary, and it is not suggested that the differences shown in the map between adjacent areas are determined with any great precision.

Fig. 2 shows the distribution of acarine disease as

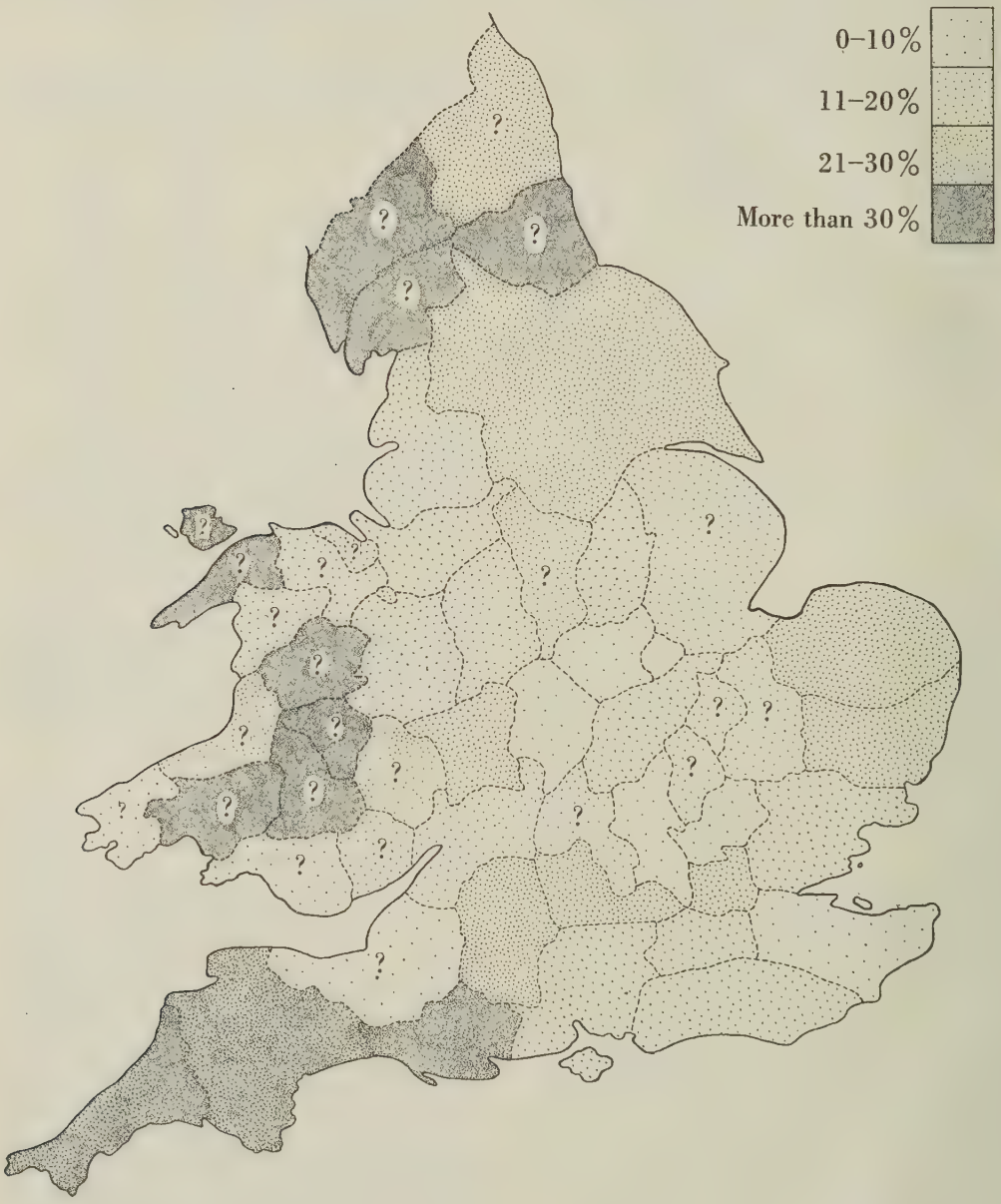


Fig. 1. The incidence of acarine disease in England and Wales as determined by surveys conducted during the winters 1941–2 and 1943–4. (? indicates that less than twenty-five samples were received from the county.)

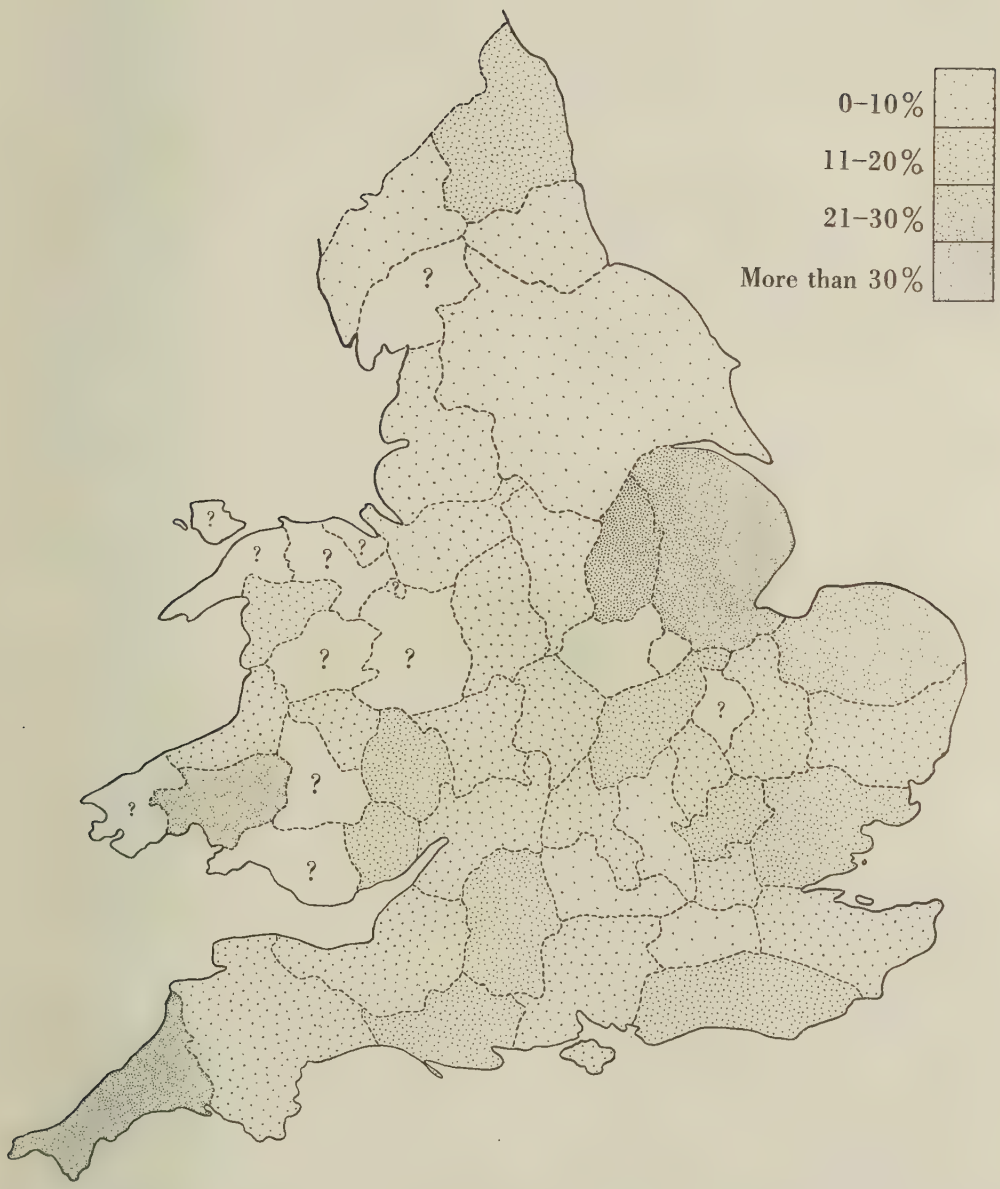


Fig. 2. The incidence of acarine disease in England and Wales as determined from samples received for routine examination during the period Jan. 1941–Oct. 1944 inclusive. (? indicates that less than twenty-five samples were received from the county during this period. On the average eighty samples were received from each county during the above period.)

determined from samples received for routine examination during the period January 1941–October 1944 inclusive.

Comparison of Figs. 1 and 2 shows that acarine disease is not clearly confined to any definite areas of England and Wales, but, generally speaking, is apparently more abundant in the West and South-West.

Of the 554 samples examined in connexion with the 1943–4 survey, 17·3% were found to be infected with acarine disease, as compared with 20·7% in the 1941–2 survey, which was based upon approximately the same total number of samples. The figure of 17·3% is probably within 3–4% of the true value, and it seems safe to conclude that the position with regard to acarine disease, taking the country as a whole, has not deteriorated since the spring of 1942, but, if anything, shows signs of improvement.

TABLE 2. *Analysis of the results of the examination of samples of adult bees received during the period Jan. 1941–Oct. 1944 inclusive*

Month	Total no samples	% with acarine	% with Nosema	% with Amoeba	% with paralysis
Jan.	139	45	4·3	0·0	0·7
Feb.	293	46	1·0	1·7	2·4
Mar.	602	41	0·7	0·3	4·0
Apr.	536	18	5·4	3·2	11·0
May	513	10	4·5	1·2	30·4
June	458	12	5·5	0·2	24·9
July	474	22	1·1	0·0	17·7
Aug.	264	23	1·1	0·8	4·5
Sept.	212	39	0·9	0·0	2·8
Oct.	393	27	0·8	0·5	2·3
Nov.	200	23	0·5	0·5	2·0
Dec.	160	34	0·6	0·0	0·6

From Table 2, which is an analysis of the results of the examination of samples of adult bees (other than those in surveys) received during the period January 1941–October 1944 inclusive, it cannot be deduced that any of the diseases enumerated therein reaches a peak in its annual cycle at any given time of the year simply because a higher percentage of samples infected with the particular disease under consideration has been received at that time of the year. There are many factors involved which influence the percentage of samples found to be infected in any given month. For example, the percentage of samples found to be infected with acarine disease is greater during the first 3 months of the year than at any other period, but this may merely be due to the fact that beekeepers may be more likely to detect the signs of disease when the population of the colony is small. Further, those of the old bees which were infected with *Acarapis woodi* the previous autumn will, by the spring, be very heavily infected indeed, and very liable to show external signs of a diseased condition by crawling, etc.

(ii) *Nosema disease*

Cases of Nosema disease were also recorded amongst the samples from both the surveys. They only numbered eight in all, indicating an infection of 0·8% of the colonies in England and Wales, a figure which is clearly not determined with any high accuracy. A much more intensive survey would be required to obtain a reliable estimate of the average Nosema infection, but it may be noted that the eight cases found were widely distributed over the country, one occurring in each of the following counties: Kent, Sussex, Hertfordshire, Somersetshire, Leicestershire, Nottinghamshire, Northumberland, and Cardigan. The conclusion that there is no limitation of Nosema disease to particular areas is supported by Fig. 3, showing the distribution of samples found to be suffering from this disease during the period January 1941–October 1944. It will be noted that there appears to be a particularly heavy area of infection in Buckinghamshire, Hertfordshire, Middlesex and Surrey, and another in Leicestershire, with yet a third in the north of England embracing Cumberland, Lancashire and Yorkshire.

Table 2 shows that the highest percentages of samples suffering from this disease are obtained during April, May and June.

(iii) *Amoeba disease*

This disease was first recognized in this country in 1929 by Morison (1931). No samples suffering from this disease were found amongst those sent to Rothamsted until the spring of 1943. Since then a number of cases (48) have been discovered. Fig. 4 shows the distribution of these cases.

It will be noted that colonies of bees suffering from this disease have been discovered in widely separated parts of the country, and there does not seem to be any reason to assume that it is not present in every county. It will be seen from Table 2 that most cases have been detected during April.

(iv) *Bee paralysis*

Many examples of colonies of bees suffering from one or another of this group of complaints (Butler, 1943) are discovered every year by means of samples of adult bees sent for examination. Table 2 shows that the greatest percentages of samples suffering from paralysis are received during May and June, and that the great majority of cases occur during those months of the year when the bees are more active. This accords well with the belief expressed by Butler (1943) that in the majority of cases paralysis is due to some form of dietary deficiency or poisoning rather than to a disease caused by some pathogenic organism.

Bee paralysis is undoubtedly present in all areas where bees are kept, though, as might be expected, many more cases occur in some areas than in others.

Fig. 3. Distribution of 160 cases of Nosema disease in England and Wales as determined from samples received for routine examination during the period Jan. 1941–Oct. 1944 inclusive. Shaded counties are those in which Nosema has been found, the number of cases found also being denoted in each case.

Fig. 4. Distribution of the 48 cases of Amoeba disease in England and Wales as determined from samples received for routine examination during the period Jan. 1941–Oct. 1944 inclusive. Shaded counties are those in which Amoeba has been found, the number of cases found also being denoted in each case.

CONCLUSION

It appears to be fairly safe to conclude that none of the diseases that have been considered above is restricted in its distribution to any particular areas in England and Wales, and it seems probable that each does in fact occur in every county. The most serious disease of adult bees in England and Wales is undoubtedly acarine disease which, although it may, as stated in the Introduction, have been confined to three counties in 1909, now occurs in every county. Fortunately, efficient methods of eliminating this disease from a colony of bees are now well known, and there do not appear at present to be grounds for anticipating any further serious spread of this disease. Rather, as beekeepers become better educated in their craft and as new and improved methods of treatment become available, we are likely to see a decrease in the incidence of this disease.

Nosema and Amoeba diseases are not yet sufficiently common to give rise to apprehension; but it is worth noting that these diseases are believed to be responsible for serious losses of bees in Europe. As no effective cure is yet known for either of them, it will be well for beekeepers to be on their guard against them and to isolate and in severe cases destroy all infected colonies.

One or more of the various forms of bee paralysis occur in every area, but since most are due to temporary dietary deficiencies or to poisoning, and are neither contagious nor infectious, the damage that they do to the beekeeping industry, although appreciable, is not really serious. The colonies of bees involved usually recover as soon as the conditions causing the complaint become changed.

I wish to thank Mr D. J. Finney of the Statistical Department, Rothamsted Experimental Station, for his advice and assistance in carrying out the two adult-bee disease surveys.

REFERENCES

BUTLER, C. G. (1943). Bee paralysis, May-sickness, etc. *Bee World*, **24**, 3–7.

FRASER, D. H. M. (1932). Skep lore. *Brit. Bee J.* **60**, 482–5.

HERROD-HEMPSALL, W. (1937). *Beekeeping, New and Old*, **2**, 1389–91. London: British Bee Journal.

MORISON, G. D. (1931). Amoebic disease of bees in Great Britain. *Bee World*, **12**, 41–2.

(*Received* 19 *March* 1945)

Soil populations of beet eelworm (*Heterodera schachtii* Schm.) in relation to cropping

BY F. G. W. JONES, M.A., *School of Agriculture, Cambridge*

(With 19 Text-figures)

Between 1934 and 1936 beet eelworm was found to be prevalent on a farm in the Isle of Ely. The virtual absence of other species of *Heterodera* and the workable nature of the soil made the farm ideal for the study of soil populations. In 1937 a sampling technique for estimating the cyst, viable cyst and egg content of soil was developed and standardized. The first part of the paper deals with the sampling technique and the investigation of the various steps of which it is composed. When put into operation, the technique gave consistent results, but difficulty was experienced with counts of viable cysts and eggs owing to bias between different observers.

The second part of the paper gives the results obtained by the application of the technique to twenty-four fields during the period 1937–42. There appears to be a correlation between the general level of the eelworm population and the frequency with which susceptible crops are grown, and also between the general level of the population and the amount of damage to the crop, 'beet sickness' being most extensive and most severe where populations are greatest. Marked increases in the eelworm population follow the planting of susceptible crops upon heavily infested land, while smaller increases occur in fields with low eelworm populations. Fields which were completely 'beet sick' required very long periods of rest from susceptible crops (more than 10 years) before the eelworm populations in them

fell to a low level and before they could be regarded as safe to grow sugar beet once more. The general effect of non-susceptible root crops is to bring about a lowering of the eelworm population. Cereals have the same general effect, but to a lesser extent. In some instances, rises were recorded after the growing of cereals. These appeared to be due to weeds, or to susceptible crops growing as weeds amongst the cereal crops. Claying had a beneficial effect.

The results of the investigation indicate the difficulties which may arise where close cropping is practised with crops susceptible to beet eelworm, and the desirability of avoiding widespread 'beet sickness' in important beet-growing areas. Brassicae are capable of increasing beet-eelworm populations, although they do not as a rule show signs of distress when growing on land where sugar beet develops the symptoms of 'sickness'.

A strong correlation was observed between the viable cyst and egg content of bulk samples. This suggests that viable cysts are a better measure of the general level of the eelworm population than might have been supposed. Cysts decay slowly and remain in the soil long after they are devoid of living contents. For this reason cysts alone are better as a guide to the past history of an infestation than as a measure of its present intensity. The eelworm population of any field is complex and has highly individual characteristics. The best measure of this population so far devised is an estimate of the encysted egg population. While this may be adequate where it is desired to note changes in the level of the population following a particular crop or treatment, it is not nearly so satisfactory where a forecast of damage to an ensuing crop is required.

In examining cysts for viability, fungal hyphae and the fruiting bodies of other organisms were found inside. One of these, a fungus of the Order *Chytridiales*, had fruiting bodies similar to microcysts (Triffitt, 1935), and it is concluded that some part at least of the microcyst population of soils is composed of the fruiting bodies of fungi of this Order. Another organism, probably a fungus, caused blackening of the eggs. This was almost certainly a parasite, and its rather high incidence suggested that it might be of importance in reducing egg populations.

In conclusion, a number of suggestions are made for the adaptation of the technique for advisory purposes. Experience suggests that the general level of the egg population above which it is unsafe to grow sugar beet or mangolds on Fen soils is about 10 eggs per g. of air-dried soil or approximately 0·1 viable cysts per g.

Introduction

In 1934 beet eelworm (*Heterodera schachtii* Schm.) was found attacking sugar beet on a farm near Chatteris, Isle of Ely (Petherbridge, 1934). Field observations and examination of soil samples in the laboratory during 1935 and 1936 showed that most of the fields on the farm were infested, some intensely. The farm lies near the edge of the peat-fen formation. The soil consists of black fen-peat mixed with varying quantities of buttery blue clay and contains very few stones. Potato and oat eelworm are virtually absent, a few empty cysts of the former having been found in one field, and a single cyst of what appears to be the latter, discovered on oats, in another field during the whole 10 years in which the farm has been under observation. The workable nature of the soil and the lack of complications due to the presence of other eelworms made the farm ideal for study of soil populations in relation to cropping. The control measures adopted by the farmer had the effect of lowering the eelworm populations. For this reason no lengthy investigation of soil-sampling technique was possible. Nevertheless, the soil-sampling technique used in 1935 and 1936 was considerably improved and standardized during 1937 and then put into operation from 1938 to 1942. The present paper deals with the soil-sampling technique and the results obtained by its use over the period 1937–42.

In early observations on the relations between eelworm numbers and 'sickness' in various crops, an attempt was made to correlate the abundance of eelworms with the degree of 'sickness' in the crop. One of the objects of these observations was to establish the pathogenicity of the eelworm. A further object was the practical one of finding a method of assessing the probability of 'sickness' by an estimation of the eelworm content of the soil. The ultimate aim of these population estimates was not so much the assessment of the population in terms of morphological units such as cysts, viable cysts, eggs or larvae, as the assessment of its 'infestation potential'. This potential is compounded of the individual potentials of each cyst, larva or egg. Each individual potential is a quantity related to the vitality of the individual cyst, larva or egg, and in particular to its capacity for infecting the normal host plant, sugar beet.

Some workers in the past have measured the 'infestation potential' of a given population by the number of cysts present in the soil. Cysts, however, vary considerably in linear dimensions and even more in cubic capacity. Further, in view of the fact that two samples of soil may contain the same number of cysts, mainly viable in one case and mainly non-viable in the other, it is clear that viable cysts are a better measure of infestation potential than cysts. Again, of two samples of soil containing equal numbers of viable cysts, one may contain many viable eggs and larvae and the other very few. Consequently, the number of viable eggs or larvae is an even better measure of the infestation potential. Finally, since there are grades of viability (freshly produced eggs having in all probability a greater

vitality than old), even the number of viable eggs and larvae is only an approximate estimate.

The first essential of any technique is that it should give a reasonably valid measure of the 'infestation potential'. The validity of the measure will depend upon the criterion adopted, the order of validity being indicated by the following list:

(1) Cysts. Least valid measure.
(2) Viable cysts.
(3) Viable cysts, weighted according to degree of fullness.
(4) Viable eggs and larvae. Most valid measure at present in use.
(5) Viable eggs and larvae, estimated by, or coupled with, a biological test of viability.

Reference to the literature on soil sampling for the estimation of populations of *Heterodera* shows that samples have been collected from the field in a great variety of ways and submitted to equally varied treatments in the laboratory. In most cases the authors did not proceed as far as the estimation of the egg population. However, Fenwick (1940, 1942) has described a laboratory technique for assessing egg populations which represents an important advance on older methods. Had his work been published in 1937, when the present study was begun, it is possible that some parts of his technique would have been adopted.

Description of technique

Field sampling

In each field under observation, permanent 2-acre plots were laid down. Within each plot about fifty points, uniformly spaced, were chosen, their exact number and distribution depending upon the plot shape convenient for any particular field (e.g. 7×7, 5×10, 4×12, 3×16). In every case the distance between points and rows of points was 14 paces. At each point, a soil boring was taken to a depth of 8 in. with a 2 in. auger, the soil from all the points in a plot being collected in a bucket to form a bulk sample.

Treatment of bulk samples

Bulk samples brought back to the laboratory were passed through three sieves, first a coarse sieve, then a 4 mm. sieve and finally a 2 mm. sieve. Only very small fractions of the fen soils under test failed to pass through the various sieves. Mixing of the bulk sample into a homogeneous whole, already partly accomplished during sieving, was completed by coning 4–5 times. After mixing, a representative fraction amounting to about a quarter of the whole, was drawn from each bulk sample and placed in a shallow tray where it remained with occasional stirring until air dry at room temperature.

Cyst flotation

For cyst flotation, ten lots of 50 g. of air-dried soil were placed in 750 c.c. conical flasks. Each flask was about half filled with water and allowed to stand for about 5 min. with occasional shaking. The flasks were then filled to the brim and left for a further 5 min., during which time cysts and debris rose to the surface in the neck of the flask. The floated material was next decanted on to a pair of sieves, the upper having 20 meshes to the inch and the lower 60. The flasks were again filled and the decantation repeated a second time. The floated material on the sieves was then washed with a jet of water and the contents of the lower sieve transferred to a filter by the same means.

Cyst collection and counting

After being allowed to dry on the filter-paper, the float of cysts and debris was either used at once or stored for a time in 70% alcohol or formalin. For cyst collection, the dry float was spread thinly and carefully into concentric grooves cut into a circular board, the width of the grooves being related to the field size of the binocular microscope in use. The board was pivoted at its centre and could be rotated under the microscope so that the grooves were systematically searched. Cysts were collected with a fine brush and placed in rows on a glass slide with a small quantity of water. After counting the cysts, each one was opened and a second count of viable cysts (i.e. those containing eggs in good condition) was made.

Counts of eggs and larvae

In order to separate the eggs and larvae from the cysts and from one another, the slide bearing them was partly covered by a second placed at right angles and pressure applied. At the same time the second slide was moved on the first with a slight rotary motion. The mass of broken cysts and eggs was then transferred by means of a fine jet of water to the container of a counting apparatus and made up to a known volume. Details of the counting apparatus are given in Fig. 1.

The use of the apparatus depends on the fact that the size of drops produced by a dropping surface is constant under constant conditions. The suspension in the container is kept thoroughly mixed by a jet of air. Drops fall from the end of the dropping tube when the air outlet is constricted by means of a tap, the rate of fall depending on the degree of constriction. To calibrate the apparatus, the average drop size must be determined, and this may be done by collecting a large (known) number of drops and weighing the quantity of water so produced. The rate of fall of the drops must be approximately the same as the rate at which the drops are collected under working conditions and must not be too slow, because, in the absence of agitation, eggs and larvae

tend to fall from suspension in the longer limb of the dropping tube. The bore of this tube should be sufficient to permit the passage of broken cysts and small particles of debris which inadvertently get into the suspension. A convenient size is approximately 2 mm., which gives a drop size of about 0·05 c.c.

Drops from the apparatus were collected on old slides (new or meticulously cleaned slides cause the drops to spread unduly). The eggs and larvae present in 50 drops were counted, and from this figure it was possible to obtain an estimate of the total number of eggs and larvae present in the batch of cysts under examination.

Variations in technique

In 1937 only one bulk sample was taken per field and sieves were not used in the flotation process.

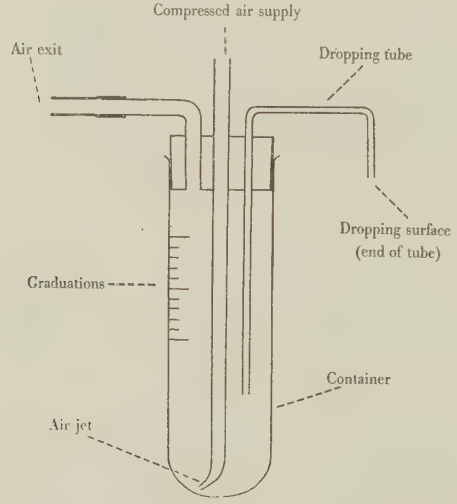


Fig. 1. Apparatus used to provide drops of approximately constant size from an agitated suspension of eelworm eggs and larvae.

Each of the ten lots of 50 g. of soil taken per bulk sample was estimated separately for cysts and viable cysts in order to provide information on the working of the technique. From 1938 to 1942 two bulk samples were taken and the 500 g. of soil withdrawn from each bulk sample were divided equally between ten flasks and the floats pooled together. Sieves were used in the flotation process, which makes the cyst, viable cyst and egg counts somewhat lower than in 1937 (see Table 5).

Analysis of technique

In 1937 a test of the validity of bulk sampling was carried out in a field with a high eelworm content (field no. 17). Two sets of samples were collected from the 2-acre plot, each equivalent to a bulk sample, but the borings were kept separate and examined independently, the whole of the soil from each boring (approx. 100 g.) being subjected to flotation. The results obtained are shown in Table 1. Altogether ninety-six borings were taken (twice 4×12), the odd numbers belonging to the first bulk sample and the even to the second.

In an ideal bulk sample all borings would yield equal weights of soil. In the test, however, the average weight of soil per boring was $102{\cdot}5 \pm 2{\cdot}15$ g. for sample 1 and $101{\cdot}7 \pm 2{\cdot}45$ g. for sample 2 (difference 0·76 g., $t = 0{\cdot}24$, insignificant). Table 2 gives a comparison of the results which would have been obtained from ideal bulk samples with those actually obtained in practice when borings are pooled together without regard to variations in weight. Table 3 compares the differences between samples 1 and 2. For each difference the value of t has been calculated according to the formula given by Fisher (1938). In no case does t exceed the expected value (the expected value of t for $n = 96$ and $P = 0{\cdot}05$ is 1·99). From the results of this test, the pooling of borings into bulk samples appears to be justified.

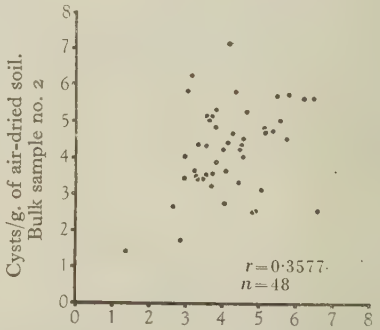


Fig. 2. Correlation between cyst counts from forty-eight pairs of soil borings taken in field 17.

Number of borings per bulk sample

In practice it is impossible to take borings from exactly the same points in the 2-acre plots on each occasion when a bulk sample is collected, for the points cannot be located accurately by pacing, especially if the field is in a rough condition. From year to year also, the soil itself is mixed and moved by ploughing and cultivations. In effect, therefore, each bulk sample consists of a series of more or less random borings drawn from approximately fifty small plots of about $\frac{1}{25}$ acre in area. On this basis further analysis was made of the data in Table 1. Before doing this, however, it seemed necessary to be assured that there was no marked correlation between the eelworm content of the pairs of borings taken in each small plot, which would suggest that the randomization within the plots was poor. Accordingly, correlation diagrams were drawn, that for cysts being given in Fig. 2. There appears to be

TABLE 1. *The eelworm content of ninety-six soil borings taken from a 2-acre plot in field 17*

No. of boring	Wt. of boring g.	Total no. per boring			No./g. of soil			No. of boring
		Cysts	Viable cysts	Eggs	Cysts	Viable cysts	Eggs	
1	78·8	250	122	9,613	3·17	1·55	121·9	1
2	62·4	390	185	30,960	6·25	2·96	496·1	2
3	79·2	289	116	8,371	3·65	1·47	105·7	3
4	91·7	465	240	20,798	5·07	2·62	227·0	4
5	107·9	411	179	19,170	3·81	1·66	177·6	5
6	102·1	545	264	22,893	5·34	2·59	224·2	6
7	100·0	429	230	19,602	4·29	2·30	196·0	7
8	101·9	478	238	29,110	4·69	2·34	285·8	8
9	103·9	387	157	7,536	3·73	1·51	72·5	9
10	97·7	350	115	9,819	3·58	1·18	100·5	10
11	105·6	473	219	23,960	4·48	2·07	226·8	11
12	78·5	315	124	13,000	4·01	1·58	165·6	12
13	100·8	360	193	13,910	3·57	1·91	137·9	13
14	99·7	344	158	11,500	3·45	1·59	115·3	14
15	94·3	455	133	17,360	4·83	1·41	184·1	15
16	69·4	245	111	12,230	3·53	1·60	176·1	16
17	112·0	338	149	14,291	3·02	1·33	127·6	17
18	103·9	606	358	22,120	5·83	3·45	212·9	18
19	110·3	563	294	24,530	5·11	2·67	222·4	19
20	92·3	449	248	33,830	4·86	2·69	366·5	20
21	101·6	364	218	19,450	3·58	2·15	191·4	21
22	87·8	450	312	31,010	5·13	3·55	353·1	22
23	81·8	108	63	4,956	1·32	0·77	60·6	23
24	68·3	97	59	5,425	1·42	0·86	79·4	24
25	77·6	254	158	11,650	3·27	2·04	150·1	25
26	108·1	391	209	20,110	3·62	1·93	186·0	26
27	94·7	543	298	27,740	5·73	3·15	292·9	27
28	105·0	475	232	23,220	4·52	2·21	221·1	28
29	94·1	451	237	38,880	4·79	2·52	413·2	29
30	93·1	331	148	28,970	3·56	1·59	311·2	30
31	87·0	543	393	21,350	6·24	4·52	245·4	31
32	96·3	544	219	38,850	5·65	2·27	403·3	32
33	97·6	338	200	19,890	3·46	2·05	203·8	33
34	90·6	310	152	13,730	3·42	1·68	151·5	34
35	95·6	364	161	18,080	3·81	1·68	189·1	35
36	106·4	415	205	14,310	3·90	1·93	134·5	36
37	86·8	466	223	18,670	5·37	2·57	215·2	37
38	81·2	387	178	14,520	4·77	2·19	178·8	38
39	104·4	426	164	19,580	4·08	1·57	187·6	39
40	82·4	228	68	8,898	2·77	0·83	108·0	40
41	97·9	346	187	25,860	3·53	1·91	264·1	41
42	98·2	424	178	17,990	4·32	1·81	183·2	42
43	72·7	300	113	13,370	4·13	1·56	184·0	43
44	100·8	447	234	14,480	4·44	2·32	143·7	44
45	94·5	548	182	13,200	5·80	1·93	139·7	45
46	90·9	524	196	16,740	5·76	2·16	184·2	46
47	90·3	400	131	9,802	4·43	1·45	108·5	47
48	112·0	372	133	13,390	3·32	1·19	119·4	48
49	104·2	310	115	18,310	2·98	1·10	175·7	49
50	96·9	392	202	19,460	4·05	2·08	200·8	50
51	86·8	332	110	8,662	3·82	1·27	99·8	51
52	140·9	683	250	25,930	4·85	1·77	184·0	52
53	125·6	567	211	17,290	4·51	1·68	137·7	53
54	96·7	419	234	16,840	4·33	2·42	174·1	54
55	120·0	667	336	32,530	5·56	2·80	271·1	55
56	113·4	569	240	27,490	5·02	2·12	242·4	56
57	107·6	481	262	21,760	4·47	2·43	202·2	57
58	112·2	507	216	16,900	4·52	1·93	150·6	58
59	102·3	424	241	25,330	4·14	2·36	247·6	59
60	95·6	683	358	29,540	7·14	3·74	309·0	60
61	92·5	306	163	12,040	3·31	1·76	130·2	61

TABLE 1 (*continued*)

No. of boring	Wt. of boring g.	Total no. per boring: Cysts	Viable cysts	Eggs	No./g. of soil: Cysts	Viable cysts	Eggs	No. of boring
62	100·4	342	182	13,800	3·41	1·81	137·4	62
63	113·2	299	176	20,580	2·64	1·55	181·8	63
64	116·0	310	153	19,720	2·67	1·32	170·0	64
65	115·5	464	248	25,630	4·02	2·15	221·9	65
66	103·1	436	211	24,230	4·23	2·05	235·0	66
67	85·4	316	144	17,200	3·70	1·69	201·4	67
68	87·3	281	141	20,040	3·22	1·62	229·5	68
69	99·8	659	396	21,890	6·60	3·97	219·3	69
70	123·5	319	163	21,000	2·58	1·32	170·0	70
71	103·2	421	188	20,370	4·08	1·82	197·4	71
72	107·7	392	317	32,730	3·64	2·94	303·9	72
73	115·2	498	285	26,020	4·32	2·48	225·8	73
74	117·5	682	348	20,000	5·80	2·96	170·2	74
75	91·7	426	176	30,740	4·66	1·92	335·2	75
76	107·2	565	337	34,133	5·27	3·14	318·5	76
77	108·9	708	326	40,020	6·50	2·99	367·5	77
78	96·9	548	273	40,640	5·66	2·82	419·5	78
79	118·5	649	243	24,470	5·47	2·05	206·4	79
80	114·5	653	300	32,160	5·70	2·62	280·8	80
81	133·3	442	213	19,300	3·32	1·60	144·7	81
82	127·7	561	293	26,610	4·39	2·30	208·3	82
83	107·0	305	150	12,180	2·85	1·40	113·8	83
84	112·6	308	168	30,400	2·74	1·49	270·0	84
85	126·6	377	153	17,490	2·98	1·21	138·1	85
86	109·0	376	186	21,030	3·45	1·71	193·0	86
87	125·8	414	222	18,990	3·29	1·77	151·0	87
88	97·6	341	150	10,000	3·49	1·54	102·4	88
89	114·5	424	239	14,500	3·70	2·09	126·7	89
90	117·5	600	219	24,670	5·11	1·87	209·9	90
91	105·8	535	246	20,310	5·06	2·32	192·0	91
92	134·7	419	202	15,460	3·11	1·50	114·7	92
93	107·4	482	244	24,440	4·49	2·27	227·6	93
94	91·2	389	127	8,320	4·27	1·39	91·2	94
95	139·2	717	305	26,310	5·15	2·19	189·1	95
96	142·4	671	255	23,080	4·71	1·79	162·1	96
COLUMN TOTALS								
Odd numbers: bulk sample 1								
48	4,919·4	20,629	9,912	937,183	200·82	96·62	9,122·1	
Even numbers: bulk sample 2								
48	4,883·2	21,028	10,089	1,022,086	206·57	99·37	10,174·8	

Note. Weights of soil refer to soil air-dried at room temperature.

TABLE 2. *Comparison of the means of the actual and ideal bulk samples*

	Actual bulk samples: No. 1	Actual bulk samples: No. 2	Ideal bulk samples: No. 1	Ideal bulk samples: No. 2
Cysts	4·19	4·31	4·18	4·30
S.E.	±0·18	±0·18	±0·16	±0·16
Viable cysts	2·02	2·07	2·01	2·07
S.E.	±0·10	±0·10	±0·10	±0·10
Eggs	190·5	209·5	190·0	212·0
S.E.	±10·3	±12·2	±10·2	±13·4

Note. Cysts, viable cysts and eggs are expressed per g. of air-dried soil.

TABLE 3. *Comparison of differences between the means of actual and ideal bulk samples and their significance*

	Cysts	Viable cysts	Eggs
Differences between actual bulk samples 1 and 2	**0·12**	**0·05**	**18·8**
t	0·47	0·35	1·18
Differences between ideal bulk samples 1 and 2	**0·12**	**0·06**	**22·0**
t	0·54	0·43	1·30

Note. Cysts, viable cysts and eggs are expressed per g. of air-dried soil.

no marked correlation ($r=0{\cdot}3577$). The corresponding diagrams for viable cysts and eggs were also drawn and the degree of scatter proved similar to that in Fig. 2, r being 0·1597 and 0·4143 for viable cysts and eggs respectively.

The further analysis of the data in Table 1 was made only for the total cyst counts on which viable cyst and eggs counts are dependent. The plots were grouped into blocks in various ways and an analysis

TABLE 4. *Standard errors per cent of mean for bulk samples composed of various numbers of borings*

No. of borings per bulk sample	No. of subplots per block	Standard error % of mean
6	8 (4×2) lengthwise	11·4
6	8 (4×2) crosswise	11·8
8	6	10·4
12	4	8·1
24	2 (lengthwise)	5·2
24	2 (crosswise)	5·9
48	1 (4×12)	3·1

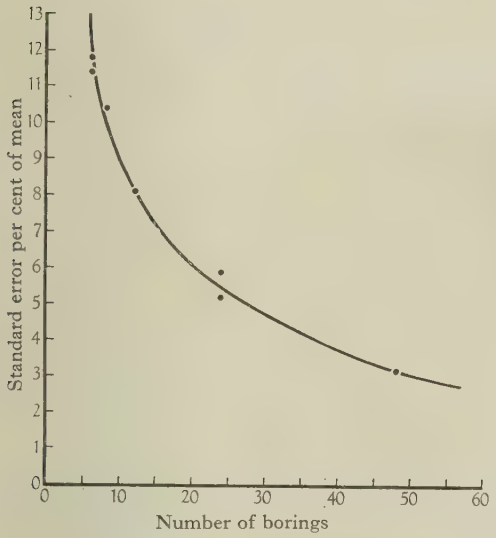


Fig. 3. The accuracy associated with bulk samples composed of different numbers of borings; the degree of accuracy being measured by the standard errors of cyst counts per cent of mean.

of variance made in each case, from which it was possible to estimate the standard errors appropriate to reduced numbers of borings per bulk sample. In Table 4 the standard errors thus obtained are expressed as percentages of the mean for bulk samples composed of the various numbers of borings. The figures are also plotted in Fig. 3, from which it may be seen that a ratio of 10% is given by about ten borings, 5% by about thirty borings, and 3% by about fifty borings. Above fifty borings, increase in accuracy is only obtained by a relatively large increase in the number of borings. It may therefore be concluded that bulk samples composed of fifty borings are satisfactory. With this number of borings the standard errors of mean for viable cysts and eggs are about 5 and 6% respectively.

Treatment of bulk samples

Thorough mixing of the bulk sample is essential in order that the portion taken for cyst flotation shall be truly representative of the whole. The fine particle size obtained by the method of treatment outlined assists in mixing, as also do the processes of sieving by which fine particle size is achieved. It was found best to treat the bulk samples in a half-dry condition; for wet soil clogs the sieves and dry soil requires considerable force to break the lumps, especially where clay is present. Excessive force is undesirable, as it might lead to the loss of cyst contents.

Samples should also be treated with the minimum of delay. Beet eelworm readily hatches from cysts under warm moist conditions even during the winter months. Soil brought in from the field may contain up to 50% of moisture. Hence the necessity for drying to an approximately standard condition, namely, air dryness at room temperature.

Cyst flotation

Tests carried out with various quantities of soil indicated that 50 g. was the largest amount which it was desirable to use in 750 c.c. flasks, and conical flasks were found to be superior to round. Weight of soil was also found better than volume on account of variability due to different degrees of packing. The whole process of flotation occupied about 20 min. If the mixture of soil and water is left for considerably longer than this, there is a danger of cysts becoming waterlogged and sinking.

As regards the total weight of soil taken for flotation from each bulk sample, the largest possible quantity is desirable so as to provide a considerable number of cysts for the viable cyst and egg counts. In practice the quantity which can be taken is limited to an amount which is convenient to handle and more especially by the quantity of floated material which can be searched for cysts without undue fatigue: 500 g. was the total quantity finally chosen. This gives some 2000 cysts from heavily infested soil, more than sufficient to provide a reliable egg count. Less reliable counts are afforded by the small number of cysts obtained from slightly infested soil.

The process of flotation described extracts some 70% of the recognizable cysts present in the soil. A further 25% can be obtained by flotation with concentrated magnesium sulphate solution, after which 5% still remain in the soil. The corresponding figures for viable cysts and eggs are: 80, 20 and 0%; and 90, 10 and 0% respectively.

Of the two sieves used in cyst flotation, the upper

retains particles above cyst size, whilst the lower permits the passage of fine particles and the smallest cysts. These small cysts, if not discarded, form an indefinite margin to cyst counts, for observers may differ in the size level at which cysts are included in, or rejected from, their counts. In order to obtain some information about the use of sieves, two sieves, of 60 and 100 meshes to the inch respectively, were used in the estimation of forty-eight routine samples. A total of 30,066 cysts was retained by the 60-mesh sieve and 3187 passed through it and were retained the actual volume of the body of the cyst by a factor (approx. 0·7) which does not vary greatly with cyst size. The figures given in Table 5 indicate that the rejection of cysts passing the 60-mesh sieve is justified.

Poisson tests of cyst and viable cyst counts

The cyst and viable cyst counts of successive lots of soil drawn from a prepared bulk sample should fit the Poisson series. Agreement with this series may be tested by calculating $\chi^2 = [\Sigma(x-\bar{x})^2/\bar{x}]$

TABLE 5. *Cyst size, egg capacity and the use of sieves of various meshes in cyst flotation*

Sieve meshes per in.	Breadth of sieve apertures mm.	Nos. of cysts retained	% of total retained		Rough estimate of egg capacity, arbitrary units	% of total egg capacity
20	(0·9 upwards)	7*	0·2		—	
30	0·57–0·7†	125	3·0		32·0	
40	0·40–0·57	1532	36·6	84·8	174·8	98
60	0·23–0·40	1895	45·2		59·2	
80	0·19–0·23	537	12·9		5·0	
90	0·16–0·19‡	78	1·9	15·1	0·4	2
>90	<0·16	10	0·3		0·04	
Totals	—	4184	—		271·44	

* Cysts smaller than sieve apertures but entangled in debris.
† The largest cysts observed were not greater than approx. 0·7 mm.
‡ Approximates to the size of cysts just bursting from roots.

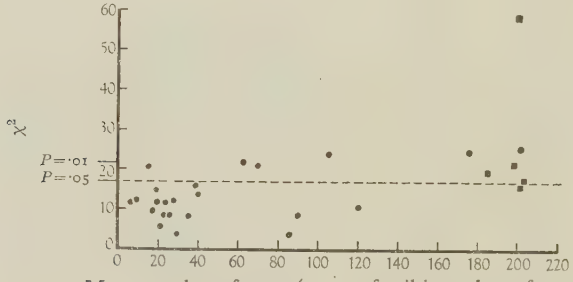


Fig. 4. Poisson tests; the values of χ^2 associated with cyst counts of the various fields for the year 1937.

by the 100-mesh sieve. The proportion of the total cysts remaining on the 60-mesh sieve was, therefore, 90%. It was also observed that most of the cysts on the 100-mesh sieve contained very few eggs. A more careful test was performed on samples of soil obtained from eight fields using sieves of various mesh size. The results obtained are given in Table 5. 98% of the total cysts were retained by the 80-mesh sieve and about 85% by the 60-mesh sieve. In column 5 of Table 5 a rough estimate of the egg capacity for each size group of cysts was obtained by cubing the mean breadth of each group (column 2) and weighting by the number in the group (column 3). In practice, the cube of cyst breadth differs from (Fisher, 1938) and comparing it with the expected value at the usual significance level ($P=0{\cdot}05$). In 1937 ten lots of 50 g. of soil were estimated from every bulk sample. The values of χ^2 thus obtained are plotted against the mean number of cysts and viable cysts per sample (500 g. in Figs. 4 and 5). These values of χ^2 apply to the whole technique from the preparation of the bulk sample up to and including the actual cyst and viable cyst counting, and cover the errors incurred at every stage. Complete agreement with the Poisson series is desirable but scarcely attainable in a technique composed of so many separate steps. The agreement observed appears reasonable under these circum-

stances. χ^2 values for high cyst counts deviate more markedly from the Poisson series than do low counts, possibly because of greater errors of cyst collection and counting due to fatigue. However, where there is a real variation between the lots (V) over and above the sampling variation, the expected value of χ^2 is $(n-1)\{V/\mu+1\}$, where μ is the mean and n the number of counts. So that if the coefficient of variation $\sqrt{V}/\mu$ remains constant (i.e. if the real relative error remains constant) over the whole range, then χ^2 would tend to increase with the increase in the number of eggs per drop. This trend is not shown in the viable cyst counts where the mean numbers per sample are much lower. These greater values of χ^2 for high cyst counts do not appear to have a very disturbing effect. Table 6 gives

TABLE 7. *Estimation of successive dilutions of a concentrated egg suspension by the drop counting method*

Degree of dilution of original suspension ...	1·0	0·9	0·8	0·7	0·6
Av. no. of eggs per drop					
Observed	33·7	27·8	24·7	23·5	20·0
Calculated	31·5	28·4	25·2	22·0	18·9
Degree of dilution of original suspension ...	0·5	0·4	0·3	0·2	0·1
Av. no. of eggs per drop					
Observed	16·7	12·7	9·7	6·9	2·9
Calculated	15·8	12·6	9·5	6·3	3·2

pooled to obtain the best estimate after weighting each according to the inverse of its variance. Errors

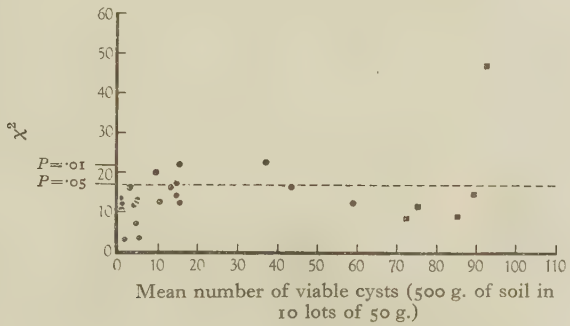


Fig. 5. Poisson tests; the values of χ^2 associated with viable cyst counts of the various fields for the year 1937.

TABLE 6. *Results of five bulk samples from field 17*

Sample no. ...	1	2	3	4	5
Cysts per g.	4·00	4·16	4·07	3·70	3·99
Viable cysts per g.	1·45	1·79	1·71	1·51	1·45
Eggs per g.	217	218	203	181	198

the results obtained from the five high counts in Figs. 4 and 5 which are plotted thus ■. These were obtained from five independent bulk samples drawn from the same field (field 17), which was also the field used for the test of bulk sampling (cp. Table 2).

Counts of eggs and larvae

Before putting the egg-counting apparatus into use, it was tested by estimating successive dilutions of a concentrated egg suspension obtained from cysts in the manner described. Table 7 gives the results of this test in which agreement between observed and calculated values will be noted over the whole range. In obtaining the calculated values, the best mean from the whole series of counts was used. This was computed by scaling up the means and variances of the counts from each dilution to correspond to a dilution of 1. The means were then due to the act of diluting the original suspension were disregarded.

After the apparatus had been in use for some time, a further investigation of the method was made using seventy-four counts; twenty-four by one observer, twenty-four by another and twenty-six with each observer counting approximately half of each batch of drops. As with haemocytometer counts, the drop counts should fit a Poisson series. In Fig. 6 the results of χ^2 tests for the seventy-four counts are shown. Deviations of $\sqrt{(2\chi^2)}$ from $\sqrt{(2n-1)}$ are plotted against the mean number of eggs per drop. Significant deviation from a Poisson series at the 5% level is indicated by values of $\sqrt{(2\chi^2)}-\sqrt{(2n-1)}$ greater than $+1{\cdot}645$, and at the 1% level by values greater than $+2{\cdot}33$. Fifty-one counts fall below the 5% level against seventy expected, fourteen below the 1% level against three expected and nine beyond the 1% level against one expected. The excessive number of counts falling between the 5 and 1% levels of significance may be due to errors of counting and the adhesion of eggs in twos and threes which cannot be entirely eliminated. Investigation of the nine counts falling beyond the 1% level showed that these marked

deviations of $\sqrt{(2\chi^2)}$ were due to failure to separate egg masses or to express eggs from the cysts. Seven of these anomalous counts were made by one observer, which shows the necessity for care with this step in the technique. In seven of the anomalous counts also, a single large drop count out of the batch of fifty was responsible for the abnormally high value of $\sqrt{(2\chi^2)}$. When the anomalous counts were neglected, the values of $\sqrt{(2\chi^2)}$ were considerably reduced, in some cases below significance. Abnormally large drop counts have the greatest effect upon the mean when the number of eggs per drop is small or when only a few drops are counted. Hence the desirability of counting a large number of drops and of working with fairly concentrated suspensions. Referring again to Fig. 6, it will be noted that the deviations from the Poisson series become more marked in drop counts having more than twenty eggs per drop. It has already been pointed out in the similar test performed upon cyst and viable cyst counts, that even if the real relative error remained constant over the whole range, the value of χ^2 would be expected to increase with an increase in the magnitude of the counts. This may be partly responsible for the more marked deviation with the higher drop counts. However, increased error in counting the crowded drops may also be a contributory factor. To avoid such errors, container size was adjusted, as far as possible, so as to work with suspensions giving from ten to twenty eggs per drop. Nothing in the tests performed on the drop-counting apparatus suggested that the mixing of the suspension was at fault, nor were there any abnormally low values of χ^2 which would suggest competition within the drops.

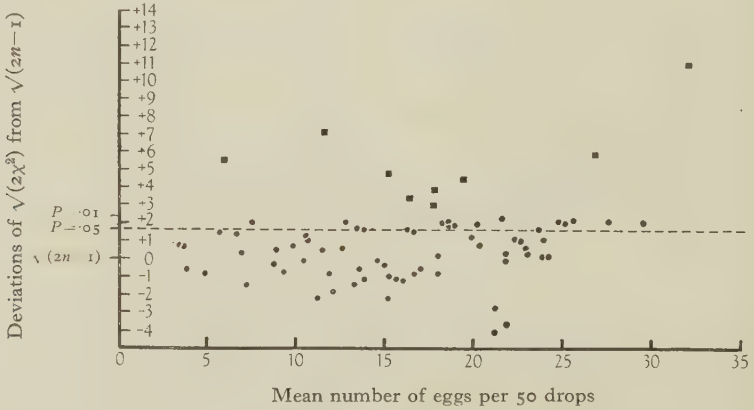


Fig. 6. Poisson tests; the values of $\sqrt{(2\chi^2)}$ associated with sets of fifty drop counts of eggs and larvae. Counts showing marked deviation thus: ■.

Overall efficiency of technique

Some idea of the overall efficiency of the technique can be obtained from the degree of correlation between pairs of estimates from each field between 1938 and 1942. These correlations are plotted in Figs. 7–9 and further analysed in Table 8, according to the observers operating the technique each year. The consistency of the results is shown by the correlation coefficient and bias between observers by the regression coefficient and the slope of the regression line. It is unfortunate that the results from observers A and B were not separately recorded in 1938 and 1939, when either observer did sample 1 or 2 indiscriminately. The regression coefficients and angles of slope are calculated for the regression of sample 2 upon sample 1. Except for the estimates of eggs obtained by eye, the correlation coefficients are very high throughout, indicating a high level of consistency. However, bias between observers in the years 1940, 1941 and 1942 was quite marked, especially with viable cyst and egg counts. This bias between observers is one of the main causes of scatter in the correlation diagrams (Figs. 7–9). Assuming that observer A was consistent in 1940, 1941 and 1942, it would be possible to use his estimates as a standard and correct those of observers B, C and D against them, thus obtaining improved total correlations and a much reduced estimate of pooled experimental error based on the differences between pairs of results. It is doubtful, however, if this assumption could be justified, as there is no guarantee that any one observer does remain consistent from year to year.

In practice much of the bias between observers arises in deciding what constitutes a viable egg or

larva. The condition of eggs and larvae obtained from cysts varies considerably and, in the counts, only those eggs which contained white opalescent protoplasm or a coiled larva in good condition were included. Discoloured or badly damaged eggs, or those held together by fungal hyphae, were not counted. In the same way discoloured or obviously damaged larvae were also rejected. Storage for a short period in 70% alcohol or formalin did not appear to have harmful effects. Viable cysts were defined as those containing one or more eggs or larvae in good condition as indicated above. No difficulties were experienced with cysts.

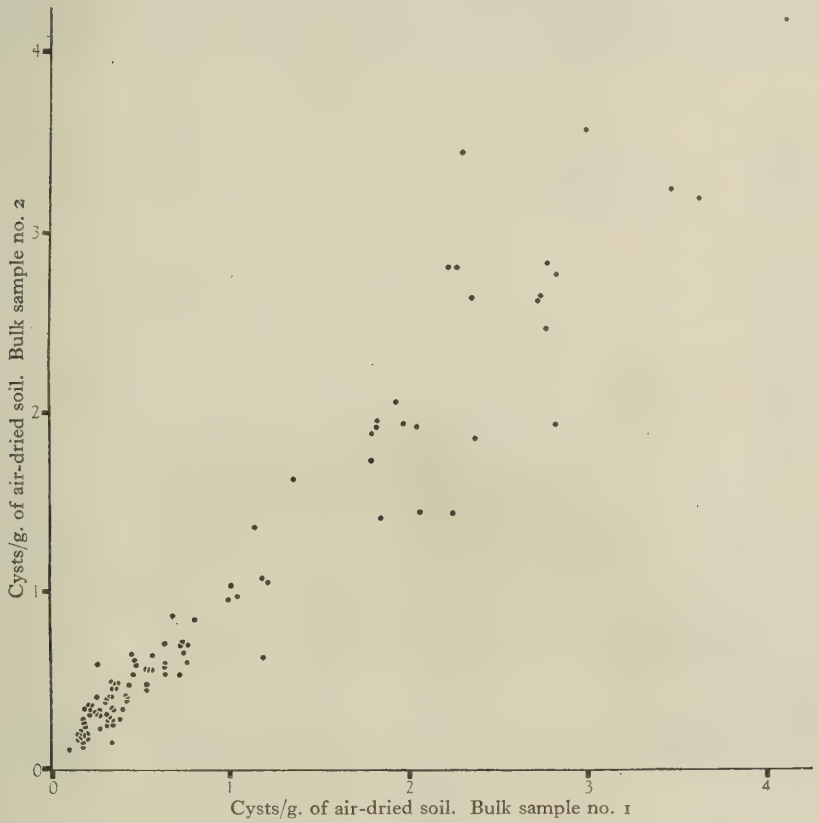


Fig. 7. Correlation between paired cyst counts obtained from twenty-four fields over a period of 5 years, four observers participating.

Results

Table 9 and Figs. 10–13 give the results obtained by the application of the technique to twenty-four fields during the period 1937–42. Bulk samples were collected from the 2-acre plots laid down in each field during the winter months of each season, at which time the numbers of free larvae in the soil are probably at a minimum. Pairs of bulk samples were collected from 1938 to 1942, whereas in 1937 only single bulk samples had been taken. Records of the crops grown were kept over the whole period, and these have been added to Table 9 and Figs. 10–13. The crop given in each year is the one which immediately preceded the estimates of the eelworm population. Fields 1–22 formed part of a Fenland farm, while fields 23 and 24 belonged to another in a different locality.

The paired estimates of the eelworm populations, which were obtained between 1938 and 1942, make possible the calculation of a pooled estimate of the experimental error against which the significance of differences in the eelworm population from year to year may be judged. The estimates for cysts, viable cysts and eggs all cover very wide ranges and the experimental error appropriate to low populations

might not hold good for high populations. The collective test devised by Bartlett (1937) was therefore applied. In this test a value of χ^2 is obtained which may be used to decide the validity of pooling:

$$\chi^2 = n \log_e S^2 - \Sigma(n_r \log_e S_r^2)$$
$$= 2{\cdot}3026 \{n \log_{10} S^2 - \Sigma(n_r \log_{10} S_r^2)\},$$

where n = the total number of degrees of freedom for the pooled estimate of experimental error;

$n_1, n_2, \ldots, n_r$ = the number of degrees of freedom available for the estimation of experimental error in each group; k being the number of groups;

found necessary to exclude all counts made by eye (i.e. not by the drop-counting method). This removed entirely the egg estimates from field 10. Although fields 18, 19, 22 and 24 have high cyst counts and are rather heavily infested (see Table 9, and Figs. 10 and 13), the only pairs of egg counts available from them are low. These therefore were included with the other fields having low counts. The remaining fields (nos. 17, 20, 21 and 23) form a satisfactory grouping on their own. The significant differences given in the last column of Table 10 were obtained from the values of S^2 for each grouping.

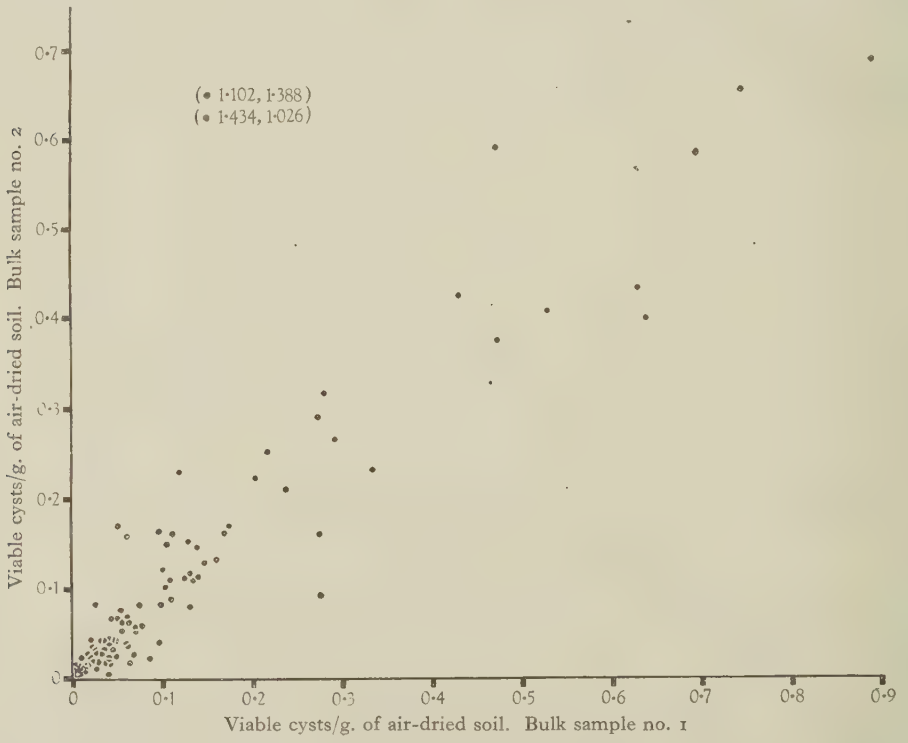


Fig. 8. Correlation between paired viable cyst counts obtained from twenty-four fields over a period of 5 years, four observers participating.

$S_1^2, S_2^2, \ldots, S_r^2$ = the variance ascribable to experimental error in each group;

$S^2 = \dfrac{n_1 S_1^2 + n_2 S_2^2 + \ldots + n_r S_r^2}{n}$, the pooled estimate of experimental error variance.

The results of the application of Bartlett's collective test are summarized in Table 10. It was not permissible to pool all the estimates of experimental error in the case of cysts, viable cysts or eggs. For cysts it was legitimate to pool high counts and low counts separately, while for viable cysts only the lowest counts could be pooled. For eggs it was

S^2 may be expressed in terms of the differences between pairs, for $S^2 = \dfrac{\Sigma D^2}{2} \times \dfrac{1}{n}$, where D is the difference between pairs and n the number of pairs. The corresponding standard error is $\text{S.E.} = \sqrt{\dfrac{\Sigma D^2}{2n}}$, and a significant difference between the means of any two pairs of estimates is $\dfrac{\text{S.E.}}{\sqrt{2}} \times \sqrt{2} \times t$, or $2 \times \text{S.E.}$, where n is large and t is approximately 2. In Figs. 10–13, the means of pairs are plotted as points,

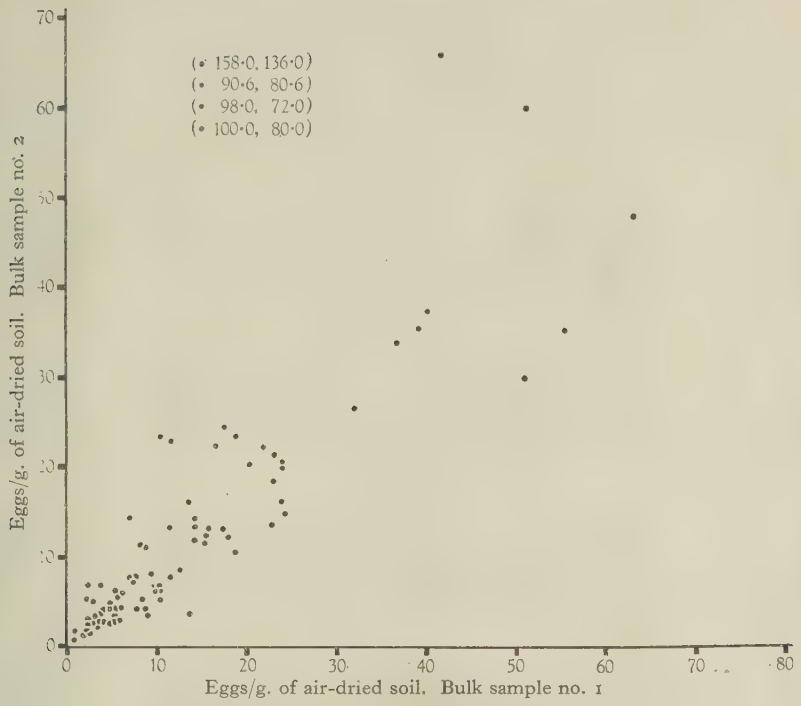


Fig. 9. Correlation between paired egg counts obtained by the drop counting method from twenty-four fields over a period of 5 years, four observers participating.

TABLE 8. *Correlation between estimates of the eelworm population obtained from pairs of bulk samples*

Year	No. of fields	Regression coefficient	Angle of slope	Correlation coefficient	Significance	Observers (A, B, C and D)
			Cysts			
1938	22	0·9651	44°	0·9893	+*	A and B doing either sample
1939	22	1·0384	46°	0·9894	+	indiscriminately
1940	23	1·1150	48°	0·9896	+	Sample 1 by A, sample 2 by B
1941	17	0·6591	33½°	0·9878	+	Sample 1 by A, sample 2 by C
	5	0·8850	41½°	0·9687	+	Sample 1 by A, sample 2 by A
1942	16	1·0047	45°	0·9841	+	Sample 1 by A, sample 2 by D
1938–42	105	1·0126	45½°	0·9766	+	A, B, C and D
			Viable cysts			
1938	22	0·9076	42°	0·9392	+	
1939	22	0·9999	45°	0·9815	+	
1940	23	0·7938	38½°	0·9553	+	As for cysts
1941	17	0·8157	39°	0·9908	+	
	5	0·8271	39½°	0·9815	+	
1942	15	0·6606	33½°	0·9598	+	
1938–42	104	0·8697	41°	0·9527	+	A, B, C and D
			Eggs			
1938	13	0·8547	40½°	0·9665	+	
1939	16	1·0046	45°	0·9855	+	
1940	20	0·7410	36½°	0·9708	+	As for cysts
1941	16	0·7511	37°	0·9873	+	
	3	—	—	—	—	
1942	16	0·6397	32½°	0·9547	+	
1938–42	84	0·8360	40°	0·9637	+	A, B, C and D
1938–42	12	0·7387	36½°	0·8261	+	Various (by eye only)†

*Significance judged from the table given by Fisher (1938). † Not included in Fig. 9.

TABLE 9. *Estimates of the eelworm population obtained from bulk samples taken in twenty-four fields over a period of 6 years*

Year	Crop	Cysts/g. air-dried soil			Viable cysts/g. air-dried soil			Eggs/g. air-dried soil		
		Sample 1	Sample 2	Mean	Sample 1	Sample 2	Mean	Sample 1	Sample 2	Mean
					Field 1					
1937	Beet	0·572	—	—	0·086	—	—	17·9	—	—
1938	Wheat	0·464	0·608	0·536	0·060	0·158	0·109	15·3	12·4	13·9
1939	Chicory	0·344	0·492	0·418	0·040	0·020	0·030	10·1	6·0	8·1
1940	Barley and oats	0·422	0·414	0·418	0·025	0·082	0·054	8·2	11·3	9·8
1941	Potatoes	0·276	0·228	0·252	0·040	0·022	0·031	4·7	3·7	4·2
1942	Wheat	0·180	0·282	0·231	0·020	0·018	0·019	8·6	4·2	6·4
					Field 2					
1937	Potatoes	0·806	—	—	0·086	—	—	7·1	—	—
1938	Carrots	0·752	0·598	0·675	0·060	0·038	0·049	5·3	6·2	5·8
1939	Carrots	0·568	0·638	0·603	0·044	0·042	0·041	2·9*	2·2*	2·6
1940	Wheat	0·569	0·567	0·568	0·067	0·026	0·047	5·9	2·9	4·4
1941	Potatoes	0·338	0·410	0·374	0·008	0·008	0·008	0·6*	1·3*	1·0
1942	Chicory	0·268	0·594	0·431	0·012	—	—	0·6	0·7	0·7
					Field 3					
1937	Wheat	0·308	—	—	0·080	—	—	10·3	—	—
1938	Potatoes	0·190	0·230	0·210	0·062	0·016	0·039	3·2	0·7*	2·0
1939	Beet	0·300	0·374	0·337	0·100	0·122	0·111	13·5	16·0	14·8
1940	Wheat	0·310	0·388	0·349	0·049	0·067	0·058	13·5	3·6	8·6
1941	Potatoes	0·202	0·200	0·201	0·032	0·028	0·030	5·3	3·4	4·4
1942	Chicory	0·156	0·176	0·166	0·012	0·008	0·010	1·8	1·3	1·6
					Field 4					
1937	Potatoes	0·382	—	—	0·008	—	—	0·1*	—	—
1938	Wheat	0·378	0·482	0·430	0·004	0·014	0·009	0·5*	1·2*	0·9
1939	Potatoes	0·180	0·146	0·163	0·002	0·004	0·003	0·0*	0·1*	0·1
1940	Wheat and chicory	0·168	0·184	0·176	0·002	0·000	0·001	0·1*	0·0*	0·1
1941	Beet	0·216	0·328	0·272	0·026	0·010	0·018	5·0	0·2*	2·6
1942	Wheat	0·256	0·408	0·332	0·020	0·018	0·019	4·0	2·9	3·5
					Field 5					
1937	Chicory	0·764	—	—	0·090	—	—	14·6	—	—
1938	Cress seed	1·032	0·970	1·001	0·334	0·234	0·284	17·5	24·6	21·1
1939	Potatoes	0·626	0·706	0·666	0·138	0·114	0·126	23·1	18·5	20·8
1940	(Non-susceptible)	0·450	0·646	0·548	0·086	0·022	0·054	11·5	23·0	17·3
1941	Wheat	0·360	0·486	0·423	0·096	0·040	0·068	7·4	7·3	7·4
1942	Chicory	0·454	0·526	0·490	0·054	0·052	0·053	5·3	4·2	4·8
					Field 6					
1937	Wheat	0·422	—	—	0·012	—	—	0·7*	—	—
1938	Beet	0·634	0·526	0·580	0·070	0·052	0·061	12·5	8·6	10·6
1939	Chicory	0·540	0·554	0·547	0·052	0·076	0·064	10·4	5·1	7·8
1940	Potatoes	0·256	0·312	0·284	0·016	0·026	0·021	4·8	4·8	4·8
1941	Beet	0·270	0·330	0·300	0·074	0·082	0·078	10·3	11·7	11·0
1942	Wheat	0·328	0·410	0·369	0·034	0·032	0·033	3·2	3·4	3·3
					Field 7					
1937	Wheat	0·586	—	—	0·084	—	—	8·9	—	—
1938	Potatoes	0·762	0·696	0·729	0·130	0·080	0·105	24·2	14·9	19·6
1939	Beet	0·730	0·710	0·720	0·168	0·162	0·165	21·8	22·2	22·0
1940	Potatoes	0·530	0·560	0·545	0·118	0·230	0·174	23·0	21·4	22·2
1941	Wheat	0·720	0·526	0·623	0·102	0·102	0·102	18·6	10·5	14·6
1942	Carrots	0·528	0·436	0·482	0·062	0·062	0·062	11·4	7·6	9·5
					Field 8					
1937	Wheat	0·380	—	—	0·040	—	—	1·5	—	—
1938	Beet	0·316	0·306	0·308	0·060	0·038	0·049	2·9	5·0	4·0
1939	Chicory	0·328	0·280	0·304	0·012	0·010	0·011	0·1*	0·0*	0·1
1940	Beet	0·346	0·450	0·398	0·050	0·170	0·110	22·8	13·6	18·2
1941	Wheat	0·188	0·334	0·261	0·046	0·042	0·044	8·4	5·3	6·9
1942	Potatoes	0·176	0·260	0·218	0·004	0·014	0·009	0·7	1·6	1·2

* Eggs estimated by eye when not in sufficient numbers to make the application of the drop counting method worth while.

TABLE 9 (*continued*)

Year	Crop	Cysts/g. air-dried soil			Viable cysts/g. air-dried soil			Eggs/g. air-dried soil		
		Sample 1	Sample 2	Mean	Sample 1	Sample 2	Mean	Sample 1	Sample 2	Mean
					Field 9					
1937	Potatoes	0·720	—	—	0·202	—	—	22·6	—	—
1938	Chicory	0·632	0·592	0·612	0·276	0·094	0·185	14·1	14·1	14·1
1939	Wheat	0·400	0·328	0·364	0·055	0·062	0·059	10·0	11·1	10·6
1940	Early potatoes	0·432	0·478	0·455	0·022	0·034	0·028	3·8	3·8	3·8
1941	Wheat	0·312	0·246	0·279	0·048	0·024	0·036	5·4	2·8	4·1
1942	Barley	0·386	0·276	0·332	0·028	0·018	0·023	9·0	3·4	6·2
					Field 10					
1937	Potatoes	0·875	—	—	0·002	—	—	0·8*	—	—
1938	Wheat	0·628	0·576	0·602	0·008	0·004	0·006	1·3	0·2*	0·8
1939	Chicory	0·168	0·170	0·169	0·004	0·002	0·003	0·1*	0·0*	0·1
1940	Beet	0·224	0·352	0·288	0·002	0·002	0·002	0·1	0·1*	0·1
1941	Wheat	0·106	0·188	0·174	0·002	0·006	0·004	0·1*	0·2*	0·2
					Field 11					
1937	Wheat	0·434	—	—	0·060	—	—	11·4	—	—
1938	Potatoes	0·344	0·348	0·346	0·032	0·042	0·037	6·0	5·8	5·9
1939	Beet	0·426	0·378	0·402	0·024	0·034	0·029	6·0	4·4	5·2
1940	Potatoes	0·220	0·304	0·262	0·026	0·028	0·027	2·3	3·0	2·7
1941	Wheat	0·248	0·320	0·284	0·040	0·044	0·042	5·7	5·6	5·7
					Field 12					
1937	Potatoes	0·516	—	—	0·190	—	—	19·4	—	—
1938	Potatoes	0·528	0·474	0·501	0·128	0·154	0·141	6·9	14·4	10·7
1939	Wheat	0·424	0·402	0·413	0·076	0·058	0·067	9·4	8·0	8·7
1940	Potatoes	0·352	0·338	0·345	0·034	0·042	0·038	4·9	2·5	3·7
1941	Chicory	0·298	0·304	0·301	0·044	0·032	0·038	3·2	2·2	2·7
					Field 13					
1937	Turnip seed	1·820	—	—	0·294	—	—	40·2	—	—
1938	Early carrots	1·266	—	—	0·180	—	—	17·5	—	—
1939	Potatoes	0·990	0·950	0·470	0·060	0·068	0·064	11·4	13·2	12·3
1940	Chicory	0·740	0·650	0·690	0·070	0·056	0·063	3·1	3·1	3·1
1941	Wheat	0·360	0·468	0·414	0·040	0·040	0·040	4·8	4·4	4·6
1942	Celery	0·474	0·584	0·529	0·040	0·018	0·029	2·0	1·5	1·8
					Field 14					
1937	Potatoes	0·168	—	—	0·016	—	—	1·3	—	—
1938	Turnip seed	0·202	0·174	0·188	0·014	0·002	0·008	0·7*	0·0*	0·4
1939	Beet	0·170	0·212	0·191	0·010	0·010	0·010	0·2*	0·4*	0·3
1940	Wheat	0·100	0·116	0·108	0·014	0·008	0·011	2·5	1·2	2·4
1941	Beet	0·340	0·140	0·240	0·018	0·024	0·021	2·4	6·9	4·7
					Field 15					
1937	Chicory	0·384	—	—	0·006	—	—	0·1*	—	—
1938	Wheat	0·210	0·356	0·283	0·000	0·002	0·001	0·0*	0·1*	0·1
1939	Potatoes	0·342	0·270	0·309	0·002	0·008	0·005	0·2*	0·5*	0·4
1940	Beet	0·147	0·175	0·161	0·010	0·024	0·017	2·3	5·1	3·9
1941	Wheat	0·176	0·124	0·150	0·004	0·000	0·002	0·1*	0·0*	0·1
					Field 16					
1937	Wheat	0·436	—	—	0·036	—	—	5·3	—	—
1938	Potatoes	0·340	0·248	0·294	0·040	0·004	0·022	5·3	0·2*	2·8
1939	Beet	0·322	0·270	0·296	0·020	0·044	0·032	3·7	6·8	5·6
1940	(Non-susceptible)	—	—	—	—	—	—	—	—	—
1941	Potatoes	0·186	0·192	0·189	0·014	0·010	0·012	2·3	2·4	2·4

* Eggs estimated by eye when not in sufficient numbers to make the application of the drop counting method worth while.

TABLE 9 (*continued*)

Year	Crop	Cysts/g. air-dried soil: Sample 1	Sample 2	Mean	Viable cysts/g. air-dried soil: Sample 1	Sample 2	Mean	Eggs/g. air-dried soil: Sample 1	Sample 2	Mean
					Field 17					
1937	Chicory	4·018	—	—	1·456	—	—	217·1	—	—
1938	Potatoes	3·610	3·204	3·407	1·102	1·388	1·245	92·0	—	—
1939	Wheat	3·452	3·264	3·358	0·620	0·732	0·676	51·0	60·4	55·7
1940	Chicory	2·283	2·460	2·872	0·430	0·427	0·429	39·0	35·4	37·2
1941	Wheat	2·822	1·946	2·384	0·628	0·570	0·599	55·4	35·2	45·3
1942	Potatoes	2·340	2·692	2·516	0·202	0·224	0·213	20·4	20·4	20·4
					Field 18					
1937	Grass	3·572	—	—	0·298	—	—	29·4	—	—
1938	Grass	2·814	2·784	2·799	0·146	0·130	0·138	10·3	23·5	16·9
1939	Grass	2·756	2·844	2·800	0·096	0·164	0·130	17·2	13·2	15·2
1940	Chicory	1·922	2·066	1·994	0·174	0·170	0·172	15·6	13·1	14·4
1941	Barley	2·056	1·442	1·749	0·108	0·088	0·098	7·4	7·3	7·4
1942	Potatoes	1·962	1·934	1·948	0·042	0·066	0·054	3·2	2·7	3·0
					Field 19					
1937	Mustard	4·072	—	—	0·312	—	—	56·5	—	—
1938	Potatoes	4·196	4·160	4·178	0·274	0·162	0·218	16·5	22·4	19·5
1939	Chicory	2·974	3·586	3·780	0·112	0·162	0·137	14·3	11·8	13·1
1940	Barley	2·206	2·811	2·509	0·130	0·118	0·124	17·9	12·4	15·2
1941	Potatoes	2·358	1·852	2·105	0·124	0·112	0·118	15·2	11·6	13·4
1942	Wheat and barley	2·714	2·638	2·671	0·098	0·082	0·090	7·9	4·1	6·0
					Field 20					
1937	Carrots	1·386	—	—	0·264	—	—	31·2	—	—
1938	Cress seed	2·726	2·660	2·693	1·434	1·026	1·230	158·0	136·0	147·0
1939	Wheat	2·036	1·926	1·981	0·744	0·658	0·701	90·6	86·6	88·6
1940	Chicory	1·778	1·884	1·831	0·694	0·588	0·641	98·0	72·0	85·0
1941	Wheat	2·244	1·430	1·837	0·890	0·692	0·791	100·0	80·0	90·0
1942	Chicory	1·812	1·964	1·838	0·638	0·402	0·570	51·0	30·1	40·6
					Field 21					
1937	Wheat	2·412	—	—	0·744	—	—	79·5	—	—
1938	Chicory	2·768	2·474	2·621	0·472	0·376	0·424	41·6	66·0	53·8
1939	Mustard undersown with grass					Field clayed				
1940	Potatoes	1·812	1·928	1·870	0·292	0·266	0·279	40·0	37·2	38·6
1941	Wheat	1·832	1·414	1·623	0·238	0·210	0·224	32·0	26·6	29·3
1942	Carrots	1·788	1·736	1·757	0·216	0·252	0·234	18·8	18·4	18·6
					Field 22					
1937	Wheat	1·732	—	—	0·314	—	—	65·3	—	—
1938	Beet	2·254	2·812	2·533	0·470	0·594	0·532	104·0	—	—
1939	Chicory and 10% beet					Field clayed				
1940	(Non-susceptible)	1·132	1·356	1·244	0·160	0·134	0·147	24·0	16·1	20·1
1941	Wheat	1·216	1·048	1·132	0·104	0·150	0·027	8·6	11·3	10·0
1942	Carrots	1·348	1·603	1·498	0·108	0·110	0·109	7·6	7·8	7·7
					Field 23*					
1937	Beet	2·355	—	—	1·234	—	—	221·0	—	—
1938	Wheat	1·184	1·072	1·128	0·528	0·410	0·464	68·2	—	—
1939	Chicory	0·800	0·834	0·817	0·138	0·148	0·143	14·2	13·4	13·8
1940	Wheat, chicory and mangolds	0·676	0·862	0·769	0·274	0·292	0·283	63·0	48·0	50·5
1941	(Non-susceptible)	0·722	0·696	0·709	0·134	0·110	0·122	24·0	20·4	22·2
					Field 24*					
1937	Beet	1·292	—	—	0·898	—	—	173·3	—	—
1938	Wheat	1·184	—	—	0·696		—	72·0	—	—
1939	Chicory	1·004	1·028	1·016	0·280	0·318	0·299	36·6	33·8	35·2
1940	Mangolds and chicory	0·892	1·188	0·908	0·628	0·436	0·532	186·0	—	—

* Fields 1–22 belonged to the same Fenland farm while fields 23 and 24 belonged to another.

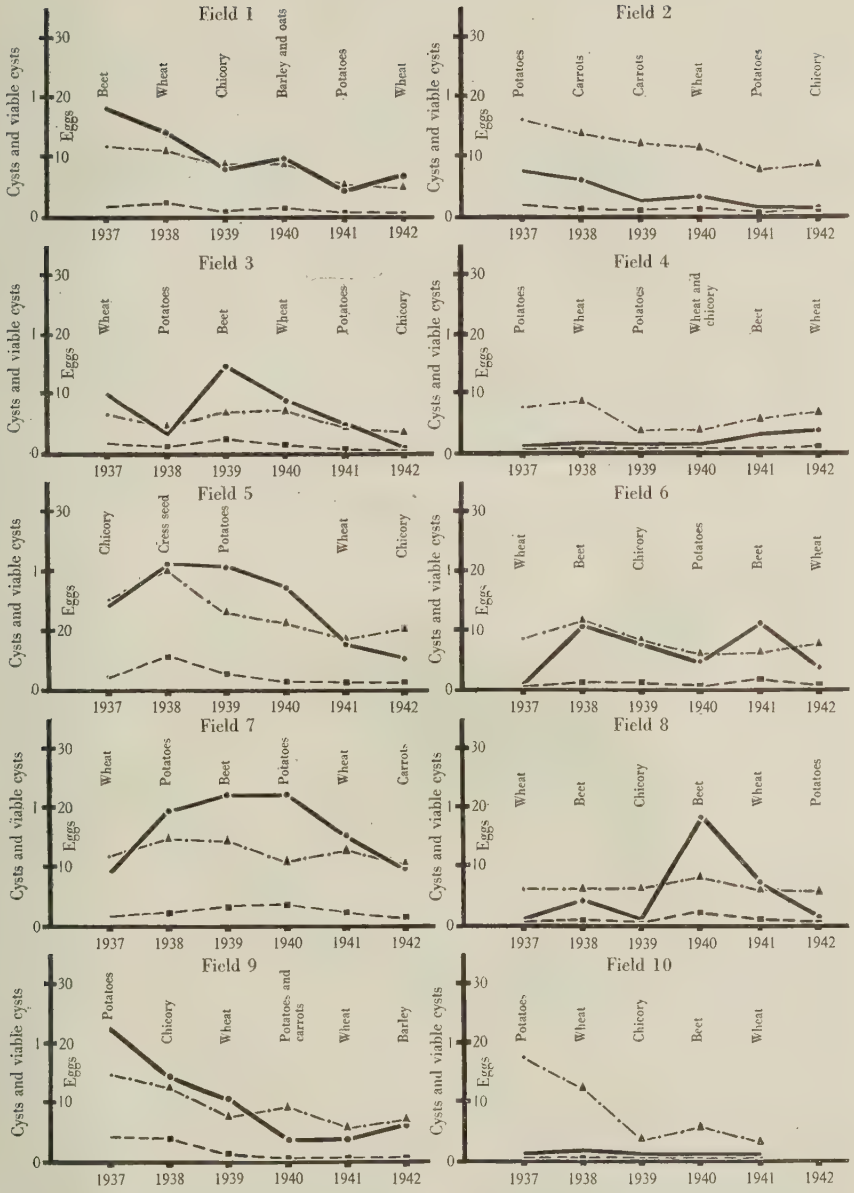


Fig. 10.

Figs. 10–13. The yearly changes in the eelworm populations of twenty-four fields. In each case the population is expressed in terms of cysts, viable cysts and eggs and the crop immediately preceding these estimates is given. Cysts ·-·-·-·-; paired estimates ▲. Viable cysts - - - -; paired estimates ■. Eggs ———; paired estimates obtained by the drop-counting method ●. With the exception of field 24, in which the egg scale has been reduced to a half, the scales are the same throughout. In fields 17 and 23, a break has been made in the egg scales in order to reduce the size of the graphs so that they will fit in with the remainder. The significance of differences between paired estimates can be judged by reference to the data given in Table 10.

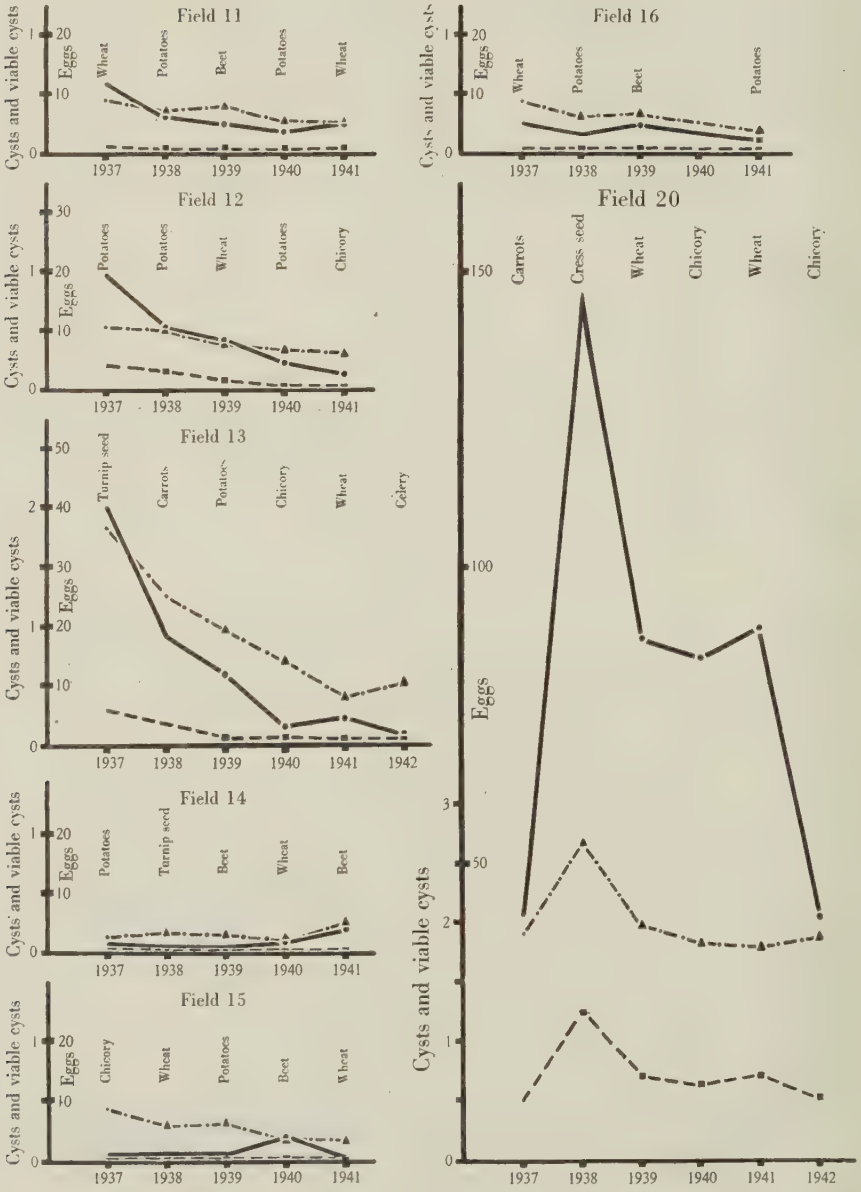

Field 11
Wheat
Potatoes
Beet
Potatoes
Wheat
Field 16
Wheat
Potatoes
Beet
Potatoes
Field 12
Potatoes
Potatoes
Wheat
Potatoes
Chicory
Field 20
Carrots
Cress seed
Wheat
Chicory
Wheat
Chicory
Field 13
Turnip seed
Carrots
Potatoes
Chicory
Wheat
Celery
Field 14
Potatoes
Turnip seed
Beet
Wheat
Beet
Field 15
Chicory
Wheat
Potatoes
Beet
Wheat
Cysts and viable cysts
Eggs
1937
1938
1939
1940
1941
1942

Fig. 11.

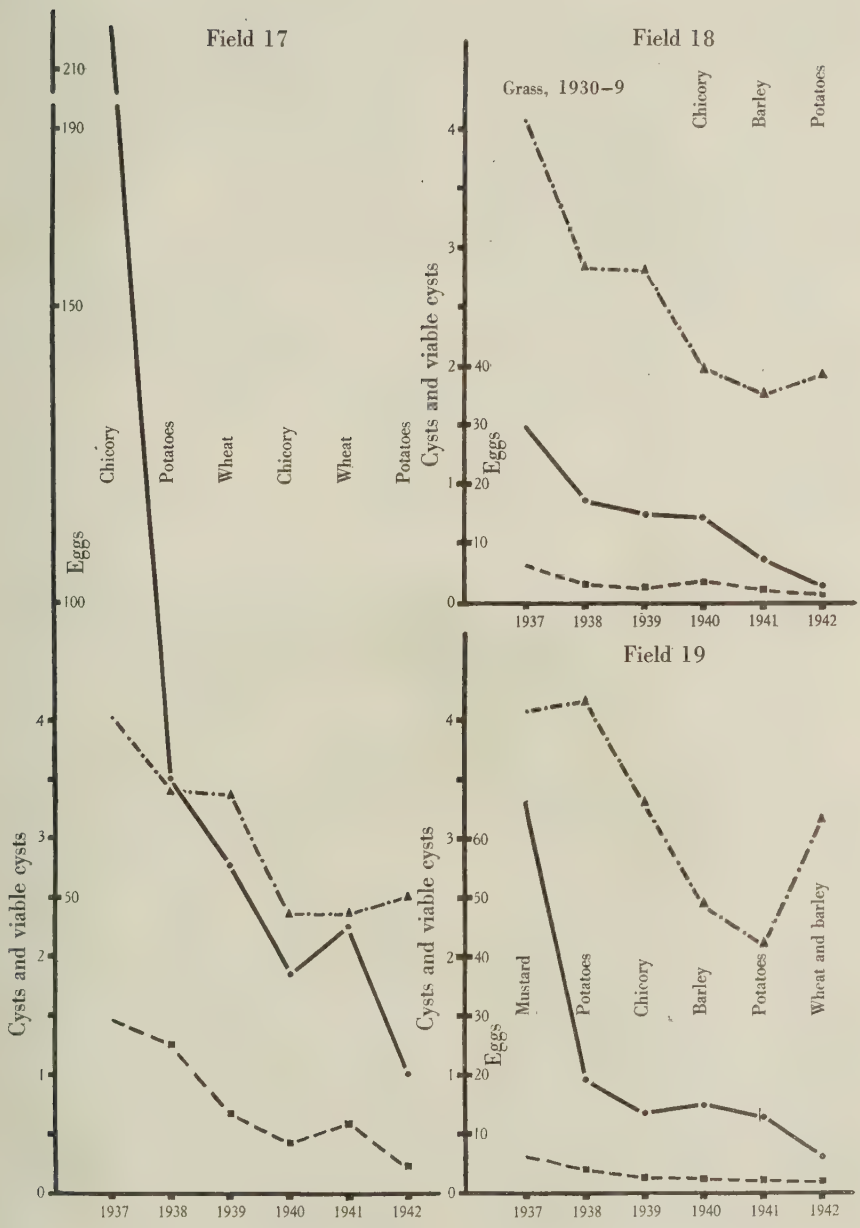

Field 17
Field 18
Grass, 1930–9
Chicory
Barley
Potatoes
Field 19
Eggs
Cysts and viable cysts
Chicory
Potatoes
Wheat
Chicory
Wheat
Potatoes
Mustard
Potatoes
Chicory
Barley
Potatoes
Wheat and barley
1937
1938
1939
1940
1941
1942

Fig. 12.

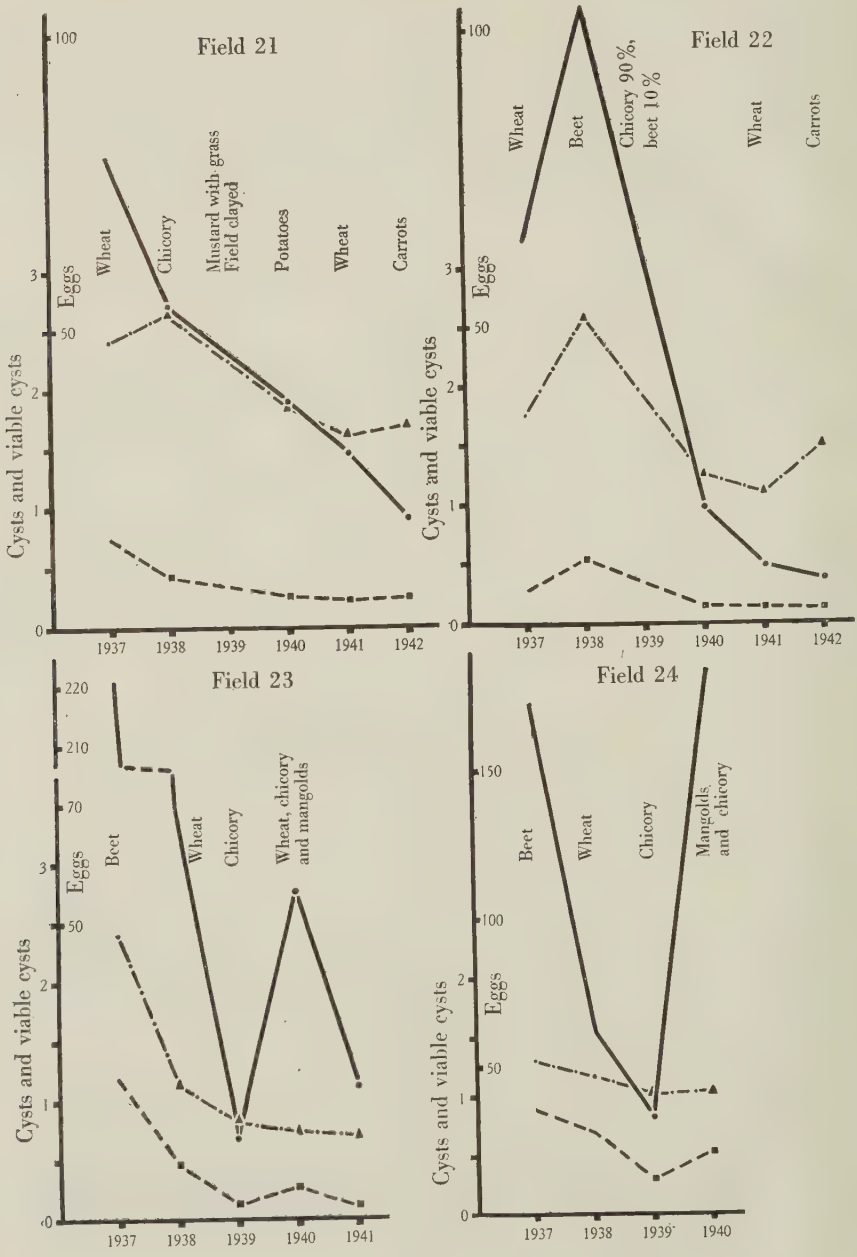

Field 21
Wheat
Chicory
Mustard with grass
Field clayed
Potatoes
Wheat
Carrots
Eggs
Cysts and viable cysts
100
50
3
2
1
0
1937
1938
1939
1940
1941
1942
Field 22
Wheat
Beet
Chicory 90%,
beet 10%
Wheat
Carrots
Field 23
Beet
Wheat
Chicory
Wheat, chicory
and mangolds
220
210
70
Field 24
Beet
Wheat
Chicory
Mangolds
and chicory
150

Fig. 13.

while single estimates and estimates of eggs by eye are represented only by inflexions in the continuous lines. It would have been possible to calculate values of significant differences appropriate to (1) the comparison of single estimates and (2) the comparison of single estimates with means of pairs. However, this was not done because the unpaired single estimates have not contributed to the pooled experimental error and because it seemed inadvisable to concede significance where only a single estimate of a parameter was available.

INTERPRETATION OF RESULTS

History of the various fields

Before proceeding to the interpretation of results, it is necessary to consider briefly the history of the fields in which they were obtained. Until 1921 certain fields, notably nos. 17–22, grew mangolds frequently because of their suitability to the crop, while the remaining fields followed for the most part, the customary Fen rotation of potatoes, wheat and oats. Detailed farm records were available from 1921 onwards, and these are summarized in Table 11. During the first period covered by the Table (1921–5), the cultivation of sugar beet was not firmly established and only one or two crops were grown on the farm. The Brassicae were black mustard, yellow turnip and cress, all grown for seed, and the non-susceptible root crops were mainly potatoes but included a few crops of carrots, while the chief cereal was wheat. During the second period covered by the table (1926–35) the sugar-beet crop almost replaced mangolds and was grown on a greater acreage. Throughout both periods, fields 17–22 were repeatedly planted with crops susceptible to beet eelworm. Thus, for example, field 20 grew sugar beet continuously from 1927 to 1930 and again in 1932. Difficulties were first experienced about 1921, when 'sick' patches began to appear in mangolds growing on fields 17, 18, 19 and 20. By 1930 field 18 was completely 'sick' and would no longer grow a successful crop of beet or mangolds; it was therefore put down to grass. Fields 17, 19 and 20, which remained arable, were but little better, and on this account they were cropped less frequently with beet and mangolds from 1930 onwards, although Brassicae continued to be grown. The farmer, unaware of the real cause of the trouble, attempted control by spreading additional quantities of farmyard manure and artificials in the 'sick' patches. However, as this did not achieve the desired result and as 'sickness' was beginning to appear in other fields, help was sought from the Advisory Centre in 1934. Examination of the farm by the Advisory Biologist between 1934 and 1936 revealed that practically every field was infested.

Acting upon the advice received from the Advisory Centre, the farmer reduced the acreage under sugar beet, mangolds and Brassicae, replacing them partly by potatoes and partly by chicory, the latter a non-susceptible crop new to the farm. The few crops of beet which were grown were placed, as far as possible, upon land where the eelworm population was low. The cropping of the farm for the period 1937–42 is summarized in Table 12, and the change of policy can be seen by comparing with Table 11.

TABLE 10. *Application of Bartlett's collective test to the results from soil samples taken between* 1938 *and* 1942

Grouping					Expected χ^2		
				Calculated χ^2	$n=k-1$		Significant
Field nos.	Description	n	k	(uncorrected)	$P=0{\cdot}05$	Significance	difference at the 5% level
			Cysts				
1–24	All fields	106	24	110·9	35·2	+	—
1–16	Fields with low counts	72	16	18·0	25·0	−	±0·133
17–24	Fields with high counts	34	8	2·4	14·1	−	±0·526
			Viable cysts				
1–16	Fields with low counts	71	16	89·9	25·0	+	—
17–24	Fields with high counts	34	8	27·2	14·1	+	—
4, 10, 14, 15	Exceptionally low counts	17	4	4·0	7·8	−	±0·010
			Eggs				
1–9, 11–16, 18, 19, 22, 24	All fields with low egg counts, omitting counts by eye	67	19	27·4	28·9	−	±5·9
17, 20, 21, 23	Fields with high counts	17	4	3·6	7·8	−	±20·0

Note. A correction may be applied to χ^2 where it has a border-line value. Correction was unnecessary for the values of χ^2 calculated above.

Susceptible crops

The general trend of eelworm populations was upwards between 1921 and 1935, the period of frequent cropping with susceptible crops. In con-

TABLE 11. *Analysis of cropping*

Field no.	1921–5 (5 years) Mangolds and beet	Brassicae	Cereals	Non-susceptible roots	1926–35 (10 years) Beet	Mangolds and Brassicae	Cereals, mainly wheat	Non-susceptible roots
1		Unknown			4	—	5	1
2	2	—	3	—	4	—	4	2
3	—	1	2	2	3	—	4	3
4	—	—	4	1	3	—	6	1
5	—	—	3	2	4	—	4	2
6	1	—	4	—	4	1	5	—
7	2	—	3	—	4	—	5	1
8	1	2	2	—	4	1	4	1
9	3	—	2	—	4	1	4	1
10	—	—	3	2	4	—	5	1
11	—	1	4	—	4	—	5	1
12	1	—	4	—	5	—	3	2
13	—	—	3	2	4	1	3	2
14	—	1	3	1	2	—	6	2
15		Cropping irregular				Cropping irregular		
16	—	—	4	1	4	1	5	—
17	2	—	2	1	3	3	2	2
18	4	—	1	—	1	2	1	—†
19	2	—	3	—	4	2	2	2
20	2	—	1	2	5	1	3	1
21	5	—	—	—	3	3	1	3
22	5	—	—	—	3	3	1	3
23*		Unknown				Frequent crops of beet		
24*		Unknown				Frequent crops of beet		
Total	30	5	51	14	76	19	78	31
Proportion	30%	5%	51%	14%	36%	9%	37%	15%
	35%				45%			

* Fields 1–22 form part of the same Fenland farm, while fields 23 and 24 belong to another farm.
† Put down to grass for the last 6 years and accounting for the remaining 3% of crops grown during the period.

TABLE 12. *Analysis of cropping for the 6-year period*, 1937–42

Crops	No.	% of total	
Beet	17	13	20
Other susceptible crops	3	2	
Susceptible crops mixed with others	7	5	
Wheat	35	26	30
Other cereals	5	4	
Potatoes	29	21	43
Chicory	21	16	
Other non-susceptible roots	8	6	
Grass	3	2	
Remainder	7	5	
Total crop/years	135	100	

Note. Further details of the cropping during this period can be obtained from Table 9 and Figs 10–13.

trast to this, the general trend was downward between 1937 and 1942, when susceptible crops were less frequently grown. This general downward trend can be observed in Figs. 10–13, particularly in the more heavily infested fields (omitting fields 23 and 24). It thus appears that there is a correlation between the general level of the eelworm population and the frequency with which susceptible crops are grown. The extent of damage to the crop (i.e. 'sickness') also appears to be correlated with the level of the population, being most severe and most extensive where the population is greatest.

Between 1937 and 1942 marked increases in the eelworm population following the sowing of susceptible crops occurred only in the heavily infested fields (nos. 1, 20, 22–24). Smaller increases followed the planting of susceptible crops on fields with low eelworm populations. In field 14 the eelworm population was so low that no obvious response occurred until three susceptible crops had been grown within the space of 4 years. Some indication of the harmful effect of growing sugar beet with only 1 year's rest from susceptible crops is shown in field 8, while the advantage of longer periods of rest can be seen in several fields, notably in the fields with high eelworm contents. In field 17 the last susceptible crop grown was cress seed in 1935. In 1937 the egg population still averaged about 200 eggs per g. of soil and had not fallen below 20 eggs per g. by 1942. Test plots of sugar beet grown in 1943 and

1944, after a rest of 9–10 years, showed that the eelworm was still decidedly active and capable of causing heavy infestations. In field 19, which had been put down to grass in 1930, following a crop of black mustard in 1929, self-sown mustard plants were present as weeds amongst the grass every year, but not in very great quantity. These plants still bore cysts in 1939. Only in 1942, after a rest of 13 years, when the egg population had fallen to less than 10/g., could the field be regarded as reasonably safe for growing sugar beet or mangolds again. Thus, where land has become completely 'beet-sick', very long periods of rest may be necessary before beet growing can be recommenced.

eelworm (*Heterodera major* O. Schm.), the brown cysts of which are indistinguishable from those of beet eelworm, and (2) the effect of weeds which grow more freely in cereals than in roots. It is clear that oat eelworm cannot be present on the farm in quantity despite the frequency with which cereals have been grown (Tables 11 and 12), for the eelworm populations show no marked responses to the cultivation of cereals as is the case with crops susceptible to beet eelworm. Further, only one lemon-shaped cyst has ever been found on cereal roots during the 10 years in which the farm has been under observation, while cysts have been found in abundance on a variety of weeds.

TABLE 13. *Summary of the effect of non-susceptible root crops on beet eelworm populations*

Crop		Significant rises	Total rises	Total falls	Significant falls
Potatoes	Cysts	—	4	18	8
	Viable cysts	—	5	17	—
	Eggs	—	4	18	2
Chicory	Cysts	—	5	13	3
	Viable cysts	—	3	14	1
	Eggs	—	—	18	2
All non-susceptible roots	Cysts	—	13	34	12
	Viable cysts	—	9	36	1
	Eggs	—	4	38	5

TABLE 14. *Summary of the effect of cereals on beet eelworm populations*

Crop		Significant rises	Total rises	Total falls	Significant falls
Wheat	Cysts	—	8	20	5
	Viable cysts	—	12	16	1
	Eggs	—	10	17	8
All cereals including grass	Cysts	1	10	23	6
	Viable cysts	—	13	22	1
	Eggs	—	13	21	9

Non-susceptible root crops

The general effect of non-susceptible root crops is to cause a fall in eelworm populations. Table 13 summarizes the changes in eelworm populations following the cultivation of these crops, of which potatoes and chicory are the most important (Table 12). The number of falls is greater than the number of rises, and in most cases the falls are of greater magnitude (see Figs. 10–13).

Cereals

The general effect of cereals is again to cause a fall in eelworm populations, but this effect is not so marked as with non-susceptible roots (Table 14). In some instances, appreciable rises in the egg populations follow the planting of cereals (e.g. field 1, 1940 and 1942; field 2, 1940; field 13, 1941; field 17, 1941; and field 20, 1941). There are two possible explanations for these rises: (1) the presence of oat

Weeds

Table 15 gives a list of weeds growing on the farm which have been found bearing cysts similar to those of beet eelworm, and there has been nothing to suggest that other eelworms were involved, except for the smaller size of the cysts in certain cases. The status of eelworms attacking weeds is receiving further attention. The most abundant weed is chickweed, which tends to form a dense mat on the surface of the soil whenever left bare and undisturbed for any length of time. Chickweed grows freely on stubbles after harvest, after early carrots or potatoes have been lifted, and whenever potato haulms die early as a result of manganese deficiency, heavy aphis attacks or other causes. The anomalies in the egg population of field 7 may possibly be due to this weed. Black mustard, yellow turnip and cress are generally scattered over the farm as weeds, but are most troublesome immediately after being grown for

TABLE 15. *Weeds on the farm bearing cysts similar to those of beet eelworm*

CHENOPODIACEAE	
Fat hen	*Chenopodium album*
Many-seeded goosefoot	*C. polyspermum*
Red goosefoot	*C. rubrum*
Common orache	*Atriplex patula*
CRUCIFERAE	
Turnip	*Brassica rapa*
Black mustard	*B. nigra*
Garden cress	*Lepidium sativum*
Shepherd's purse	*Capsella bursa-pastoris*
Treacle mustard	*Erysimum cheiranthoides*
Mithridate mustard	*Thlaspi arvense*
Hedge mustard	*Sisymbrium officinale*
Horseradish	*Cochlearia armoracia*
*Swine cress	*Coronopus ruellii*
POLYGONACEAE	
Persicaria	*Polygonum persicaria*
Curled dock	*Rumex crispus*
CARYOPHYLLACEAE	
Chickweed	*Stellaria media*
LABIATAE	
*Large-flowered hemp nettle	*Galeopsis speciosa*

* On these plants only subnormal sized cysts were found.

seed. On harvesting these crops, appreciable quantities of the seed are lost, which germinate in abundance the same autumn and during the following seasons until partly exterminated by hoeing. The effect of these plants growing as weeds in other crops, especially cereals, is shown in field 20, 1939–41 (Fig. 13), and in field 5, 1939–40 (Fig. 10). Other susceptible weeds, of which fat hen, many-seeded goosefoot, treacle mustard and shepherd's purse are the most common, are probably of less importance although they undoubtedly help to maintain eelworm populations. Charlock (*Brassica sinapis*) and wild radish (*Raphanus raphanistrum*) do not occur on the farm although they are important weeds in some Fenland areas, the latter more especially where the land is rather acid and not well suited to beet culture.

In root crops, weeds are hoed at frequent intervals, and although the early stages of the eelworm can usually be found in the rootlets of susceptible weeds, cystic forms do not always succeed in bursting the cortex before the next hoeing occurs. The effect of frequent hoeing is that of mild trap cropping.

The effect of beet tops which survive the winter and grow in the following season may be considered at this juncture. In hard winters few survive. Nor do they survive in appreciable numbers in non-susceptible root crops subject to frequent hoeing. After a mild winter, however, fair numbers can sometimes be seen in cereals. On the whole, surviving beet tops do not appear to be very important. The part which they play in perpetuating or increasing the eelworm population is probably much less important than that of self-sown potatoes on land infested with potato eelworm (*Heterodera rostochiensis* Woll.).

Claying

During the winter of 1939–40 fields 21 and 22 were clayed following the dredging of a main drainage channel which ran alongside. The 2-acre plots in each case were covered to a depth of 9–12 in. with raw clay and soil sampling was impossible. The immediate effect of thorough claying is to dilute the eelworm population of the upper layers of the soil to a very considerable extent. It probably also improves fertility. Susceptible crops were grown previous to claying on both fields, and yet the eelworm populations in the following year (1940) were not particularly high. The beneficial effect of spreading clay and spoil from dykes has been noted on a number of infested fields in the Fens.

DISCUSSION

This study had as its first aim the development of a technique which could be standardized and operated over a long period with consistent results. Standardization is the more necessary because the measure of the eelworm population obtained is purely relative. The high values of the correlation coefficients in Table 8 show that consistent results were obtained. However, the bias between observers remained a stumbling block not easily to be removed because the judgment of what constitutes a viable cyst, egg or larva is in large measure subjective. The effect of this bias was to increase the experimental error by an amount varying with different observers.

A technique based on the flotation of cysts from the soil ignores any free larvae which may be present. When soil samples are taken after susceptible crops, the eggs extruded into the bag of mucus normally exuded from the vulva of female beet eelworms are also ignored. These eggs are the first produced and probably the first to hatch; they are also unprotected, and it seems unlikely that they exert any influence after the year following a susceptible crop. Another limitation of the technique arises from the method of bulk sampling which was adopted. In Table 1, which gives the results of ninety-six separate soil borings taken over 2 acres in field 17, the eelworm population in terms of eggs ranges from as low as 60 eggs/g. of air-dried soil to as high as 496/g. This gives some idea of the great variation in the degree of infestation to be expected within a single field. The estimate of the eelworm population given by a bulk sample for this particular field would be the average level of the eelworm population without any reference to the degree of variability, namely, about 200 eggs/g.

The results of the investigation indicate the difficulties which may arise where close cropping is practised with crops susceptible to beet eelworm. If land is allowed to become completely 'beet sick', very long periods of rest from susceptible crops may be necessary before beet growing can be recommenced. The Fens of East Anglia form one of the most important beet-growing areas in the country, and are served by more beet-sugar factories than any other region. The serious consequences which might follow if any considerable acreage of land in this area became completely 'beet sick' are now clearly recognized. During the course of a survey carried out in the area (Petherbridge & Jones, 1944), heavily infested fields were distinguished from those more lightly infested. Only a few of the heavily infested fields were completely 'beet sick', and it is unlikely that the remainder will require more than 5 years' rest from susceptible crops.

The part which Brassicae can play in building up beet eelworm populations is shown in field 20 (Fig. 12), where considerable increase occurred following the planting of cress for seed. There is no reason to believe that other Brassicae, on which cysts are formed in abundance, would be less effective in this respect. Mustard, coleseed or turnips sown as catch crops after the lifting of other crops such as early potatoes and early carrots, would probably be somewhat less important than if they had been in the land throughout the growing season. No evidence for the existence of a separate species attacking Brassicae was found in the fields under study. Field 20, which gave the marked response to cress seed, had only grown one other Brassica crop between 1921 and 1937 (turnip seed in 1934), whereas seven crops of beet or mangolds had been grown during the same period besides frequent crops of mangolds in previous years.

It is interesting to note that Brassicae, although heavily attacked by beet eelworm on land which is 'beet sick', do not usually show any signs of distress. The Monthly Summaries of Plant Pests issued by the Ministry of Agriculture contain a few references to crops of Brassicae seriously affected by the attacks of cyst-forming species of *Heterodera*, but these may not be the same as the species which attacks sugar beet (Petherbridge & Jones, 1944).

When the results obtained from the application of the technique are plotted in form of correlations between cysts, viable cysts and eggs, they provide information on the relative merits of these three measures of eelworm populations. They also give some idea of the state of affairs which exists in infested soil. Figs. 14–16 give the correlation diagrams for ninety-six separate soil borings taken within the 2-acre plot of field 17. In all three diagrams there is a moderate amount of scatter and the correlation coefficients (r) are not of a very high order, showing that the relationship between cysts,

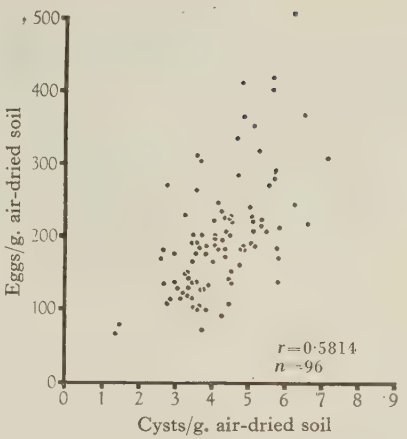


Fig. 14. The correlation between the cyst and egg content of ninety-six soil borings from field 17.

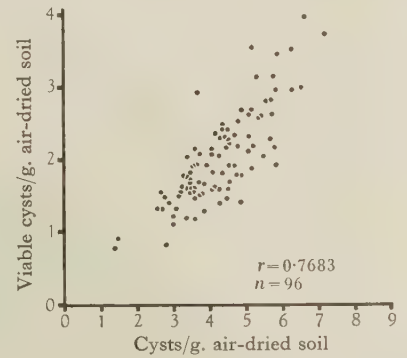


Fig. 15. The correlation between the cyst and viable cyst content of ninety-six borings from field 17.

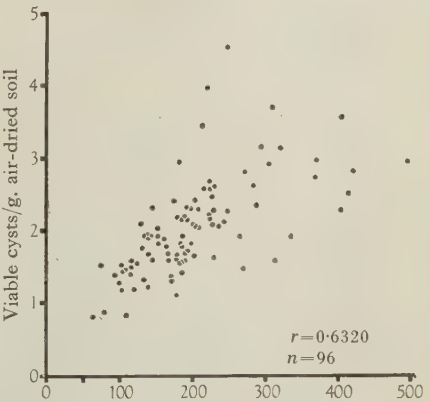


Fig. 16. The correlation between the viable cyst and egg content of ninety-six soil borings from field 17.

viable cysts and eggs is not a very close one even within part of the same field. Figs. 17–19 give the correlation diagrams for all the bulk samples taken between 1937 and 1942. Every point on these diagrams corresponds to the mean point of scatters such as those shown in Figs. 14–16. Fig. 17 shows clearly that, from field to field, cysts give a very poor indication of the egg population, 1 cyst per g. of soil corresponding to anything from 10 to 100 eggs or more and 4 cysts per g. to anything from 20 to 200 eggs. The correlation between cysts and viable cysts (Fig. 18) is also poor though somewhat stronger. The proportion of viable cysts in the bulk samples rarely exceeds one-half of the total cysts extracted by the flotation process, or one-third of the recognizable cysts in the soil, bearing in mind that flotation only extracts some 70%. In contrast with Figs. 17 and 18, Fig. 19 reveals a surprisingly high degree of correlation between viable cysts and eggs ($r = 0{\cdot}9497$). This correlation is analysed further in Table 16, where it will be noted that the high correlation holds good for different observers and different years. The regression coefficients given are those for the regression of eggs upon viable cysts. The coefficients are therefore equivalent to the mean number of eggs per viable cyst. Variation in this figure is due partly to differences in the opinion of the various observers and partly to real differences in the mean number of eggs per viable cyst from year to year.

The total correlation plotted in Fig. 19 may be said to rest on a fairly broad basis. The results from which it is derived were obtained over a period of 6 years in which four different observers operated, and from twenty-three fields, some having high and others low eelworm populations. It appears, therefore, that when a system of bulk sampling is used, viable cysts form a more satisfactory measure of the eelworm population than might have been expected. However, the period during which the results were obtained was one of falling populations. It is possible that the correlation might not hold to the same extent when populations are rising.

Close correlation between the cyst, viable cyst and egg content of soil are only to be expected during the

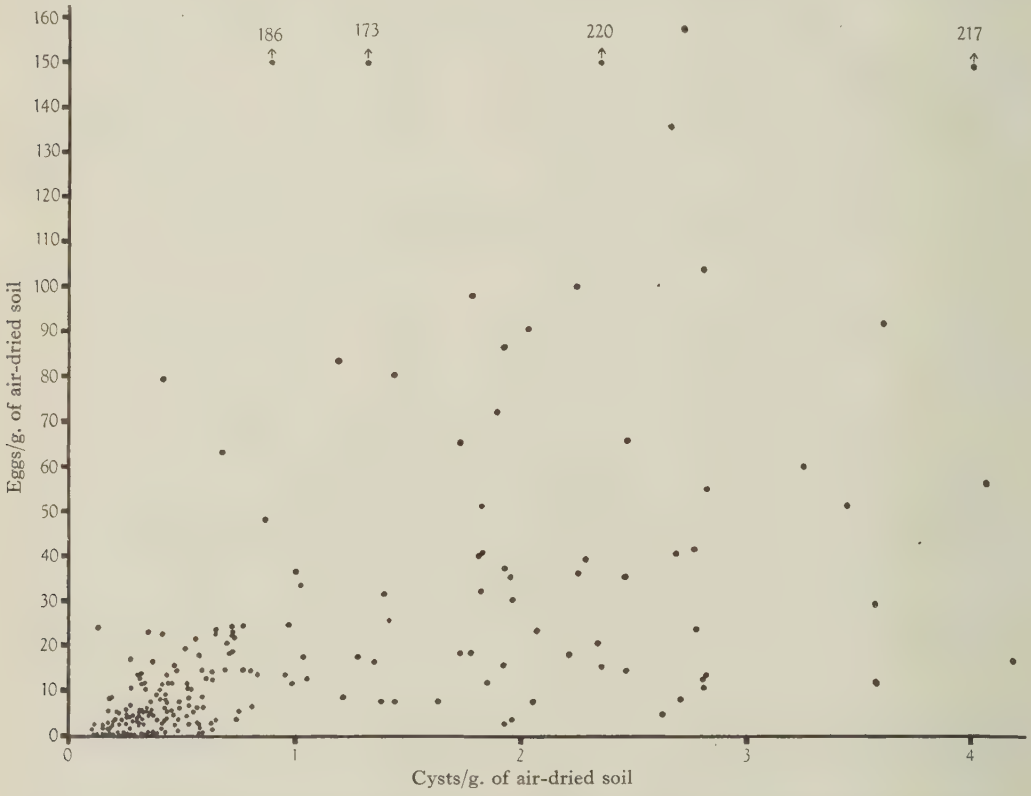


Fig. 17. The correlation between the cyst and egg content of 194 bulk samples obtained from twenty-three fields over a period of six years (omitting counts of eggs made by eye).

TABLE 16. *Analysis of the correlations between viable cysts and eggs obtained by four observers from twenty-three fields over a period of 6 years (omitting counts of eggs made by eye)*

Year	Sample no.	No. of counts	Regression coefficient*	Correlation coefficient	Significance	Observers
1937	1	19	163·4	0·9764	+	A and B doing approx. equal no. of counts
1938	1	19	98·5	0·9740	+	
	2	14	142·9	0·9784	+	
1939	1	16	100·7	0·9674	+	
	2	16	100·0	0·9489	+	
1940	1	21	191·0	0·8798†	+	A
	2	20	110·3	0·9190	+	B
1941	1	20	103·5	0·9820	+	A
	2	19	91·6	0·9330	+	C, 17 counts; A, 2 counts
1942	1	16	122·8	0·9752	+	A
	2	15	71·8	0·9801	+	D
1937–42		195	128·8	0·9497	+	A, B, C, D

* The regression is that of eggs upon viable cysts and the regression coefficient is therefore equivalent to the mean number of eggs per viable cyst.

† The low value for r is due to one large count (field 24, 1940). When this is omitted the regression coeff. = 131·2 and $r = 0{\cdot}9306$.

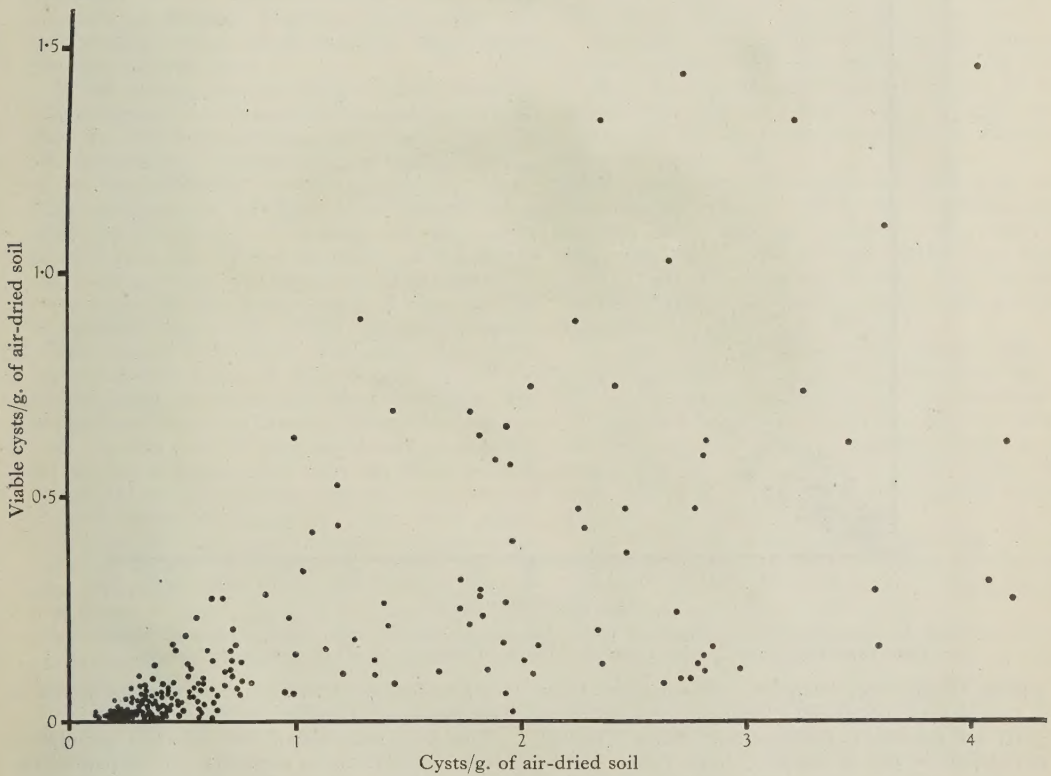


Fig. 18. The correlation between the cyst and viable cyst content of 232 soil borings obtained from twenty-four fields over a period of six years.

early stages of the build up of an infestation. With the passage of time, a preponderance of empty cysts develops. These remain in the soil as recognizable entities long after they have lost their living contents. For this reason, cyst counts are often a better indication of the past history of an infestation than a measure of its intensity at the present. With the passage of time also, the viable cysts are drawn from a complex of populations belonging to different years and possibly to different host plants. The egg population is also a complex, some being freshly produced and probably possessing greater vitality than those which have lain dormant for a time. The number of free larvae in the soil varies throughout the year and from year to year. Thus, the eelworm population of any one field develops highly individual characteristics.

The measurement of the eelworm population of soil may have two main aims:

(1) to determine changes in the level of the eelworm population following a particular crop or treatment, and

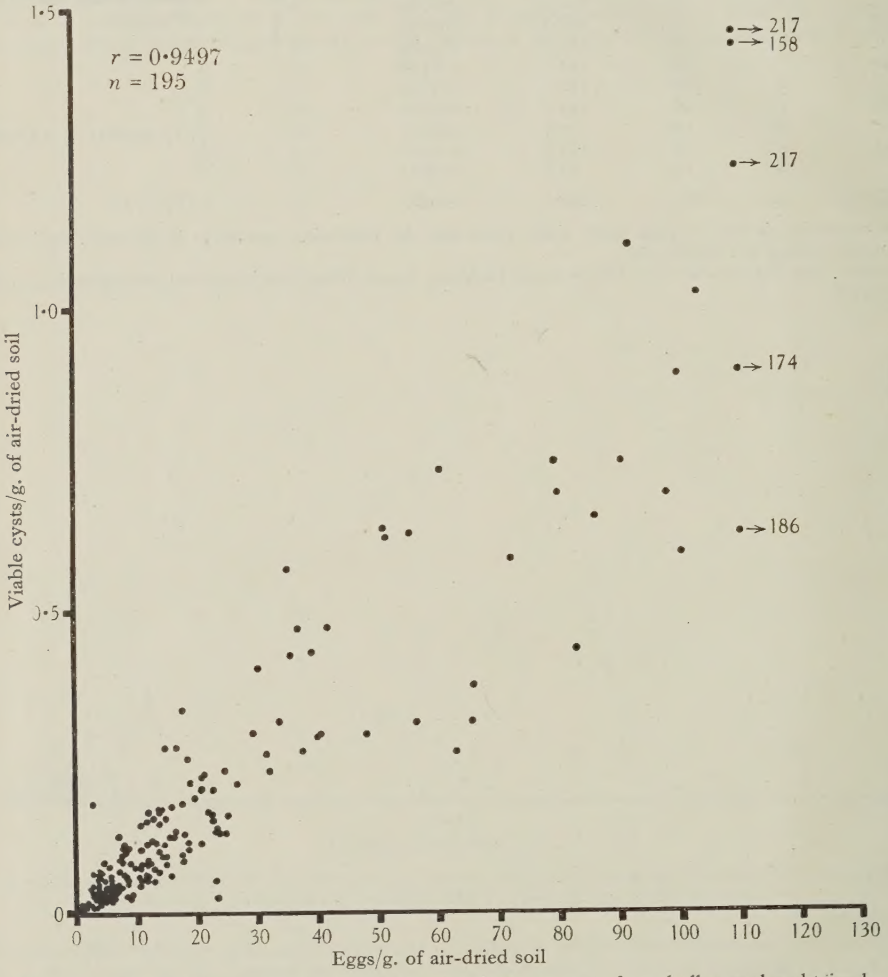


Fig. 19. The correlation between the viable cyst and egg content of 195 bulk samples obtained from twenty-three fields over a period of 6 years (omitting counts of eggs made by eye).

(2) to forecast the amount of damage which is likely to occur to an ensuing crop.

The best measure of the eelworm population which has so far been devised is an estimate of the encysted egg population. The term egg here includes those free larvae which are sometimes found within the cyst. This estimate is usually, though not invariably, obtained during the winter months when

the crop has been lifted and the number of free larvae in the soil is presumably at a minimum. Such an estimate is adequate for the first purpose named above, but not necessarily so for the second. For this purpose, what is really required is a forecast of the numbers of eelworm larvae which will be free in the soil early in the growing season. Unfortunately, the quantitative relationship between the encysted egg population and the number of free larvae which will be liberated at a later date is unknown. The problem of forecasting damage is further complicated by soil fertility and other factors.

Fenwick (1942), working upon potato eelworm, did not estimate the encysted egg population directly, but only after 'hatching' chemically with a bleaching powder solution containing 1% of available chlorine. His experiments showed that with this solution nearly all the eggs were 'hatched'. Such a method is probably free from some of the personal errors encountered by the writer in the present study. However, the number of eggs which can be 'hatched' chemically is not necessarily an indication of the number which would hatch normally in the field. Moribund larvae might also be hatched while unembryonated eggs would probably leave no trace.

While opening cysts in order to judge viability, fungal hyphae were frequently encountered within the cysts. The fruiting bodies of four organisms were also encountered. The first type was striking because of its resemblance to microcysts (Triffitt, 1935). This organism was a fungus of the Order Chytridiales and probably a member of the family Cladochytriaceae. (For a discussion of this family see Karling, 1931.) The mean size of the spherical, thick-walled fruiting bodies was 0·17 mm. and five to fourteen were found within the cysts affected. There can be little doubt that some part of the microcyst population of soils is made up of the fruiting bodies of members of the Chytridiales. The second type has so far been encountered in one cyst only. In this case the cyst was devoid of eggs and larvae, but contained more than 100 black, strongly sculptured spores similar in appearance to those of bunt of wheat (*Tilletia caries*). Some of the spores were spherical, others ovoid, the average sizes being: spherical 36 μ, ovoid 45 × 23 μ. The third type was also encountered in but one cyst. This, on opening, was found to hold more than 100 small, spherical, thick-walled spores devoid of sculpturing and measuring 16–25 μ. Some of these spores had oily granular contents while others were empty. Whereas the first three types of fruiting bodies may have been derived from saprophytic organisms, the fourth was almost certainly parasitic. It was also more commonly encountered, the incidence rising in some exceptional samples of cysts to nearly 50%. The effect of this organism, probably a fungus, was to turn the eggs black. When examined under a high-power microscope the egg membranes were seen to be filled with a mass of dark-walled spores. It is possible that this organism may be of some importance in reducing egg populations.

Application of the technique to advisory work

The method of bulk sampling is particularly suited to advisory work in which a general estimate of the eelworm population of an area of land is frequently required. For this purpose the limitations of bulk sampling are not so serious as might be supposed. If any appreciable area of a field is heavily infested, the fact will be reflected in the high cyst, viable cyst and egg counts obtained from the bulk sample. If, however, only a relatively small area is heavily infested (one boring in fifty), the estimates for the bulk sample will not be appreciably affected. On this account a farmer might be advised to grow beet only to find that part of the field was a failure. The area of the failure would nevertheless be small and, for practical purposes, the advice given, sound.

Experience with the operation of the technique in routine advisory work was obtained by using it on parts of the experimental fields not covered by the 2-acre plots, and by using it on other fields elsewhere. The results of this work lead to the following tentative conclusions:

(1) Satisfactory results may be obtained by distributing the borings more or less at random over 5 acres. Where fields are of greater size it is best to split them up and take one bulk sample from every 5 acres. Fewer borings may be taken, but if the field has to be covered it is almost as easy to take approximately fifty as some lesser number.

(2) The quantity of soil used for flotation may be reduced to 200 g. (air dried). This reduces the labour of cyst collection and counting and also the number of manipulations. With this smaller quantity of soil the apparatus described by Fenwick (1940) has no advantage over ordinary conical flasks which also give rather less debris in the float owing to the absence of upward water currents.

(3) No relaxation in the thorough mixing of the bulk sample can be allowed. It is essential that the portion of the bulk sample used for flotation should be truly representative of the whole.

(4) The level of the egg population above which it is risky to grow sugar beet or mangolds on black fen soil is about 10 eggs/g. of air-dried soil or approximately 0·1 viable cyst/g. (Fig. 19). These figures have been arrived at by trial and error and may require modification in the light of further experience.

In view of the correlation noted between viable cysts and eggs when a system of bulk sampling is used (Fig. 19), it is probable that for advisory work

the general level of eelworm populations might be conveniently estimated in terms of viable cysts. For this purpose the definition of a viable cyst might possibly be improved if only such cysts as contained a reasonable number of eggs or larvae in good condition were included. In drawing up definitions, however, the possibility of bias between observers must be borne in mind and every effort made to reduce it to a minimum.

It is not suggested that the level of the eelworm population is the only factor which enters into the development of 'beet sickness'. The adviser will, of course, take into account the past history and other details of any field in question, noting especially fertility and the performance of previous crops of sugar beet or mangolds, and will modify his method of sampling and his advice accordingly. For example, in a field where eelworm damage to the last beet crop was patchy, it might be possible to sample the patches separately from the remainder of the field and to give them longer periods of rest from susceptible crops.

The work was initiated and carried out under the general direction of Mr F. R. Petherbridge, to whom the writer is indebted for many helpful suggestions. The writer also wishes to express his gratitude to Dr D. Price Jones who collaborated in the development of the drop-counting apparatus, assisted in the collection of bulk samples, and acted as observer between 1937 and 1940. Thanks are also due to K. P. Humphries and B. D. Wragge Morley for acting as observers and for their technical assistance, to Dr J. O. Irwin for help with statistics, to Q. A. Geering for the examination of cysts containing fungi and other organisms, and to Mrs M. G. Jones for reading the script. The writer is particularly indebted to the late J. B. Allpress of Chatteris, Isle of Ely, on whose farm most of the investigations were made.

REFERENCES

BARTLETT, M. S. (1937). Properties of sufficiency and statistical tests. *Proc. roy. Soc.* A, **160**, 268.

FENWICK, D. W. (1940). Methods for the recovery and counting of cysts of *Heterodera schachtii* Schmidt. *J. Helminth.* **18**, 155.

—— (1942). The degree of *Heterodera* infectivity of soil and its determination. *J. Helminth.* **20**, 50.

FISHER, R. A. (1938). *Statistical Methods for Research Workers*, 7th ed. Edinburgh.

KARLING, J. S. (1931). Studies in the Chytridiales. VI. The occurrence of a new species of *Cladochytrium* in the cells of *Eriocaulon septangulare*. *Amer. J. Bot.* **18**, 526.

PETHERBRIDGE, F. R. (1934). Beet eelworm or beet sickness present in England. *J. Minist. Agric.* **41**, 825.

PETHERBRIDGE, F. R. & JONES, F. G. W. (1944). Beet eelworm (*Heterodera schachtii* Schm.) in East Anglia, 1934–43. *Ann. appl. Biol.* **31**, 320.

TRIFFITT, M. J. (1935). On cyst-like bodies, resembling cysts of *Heterodera schachtii*, of common occurrence in British soils. *J. Helminth.* **13**, 59.

(*Received* 22 *March* 1945)